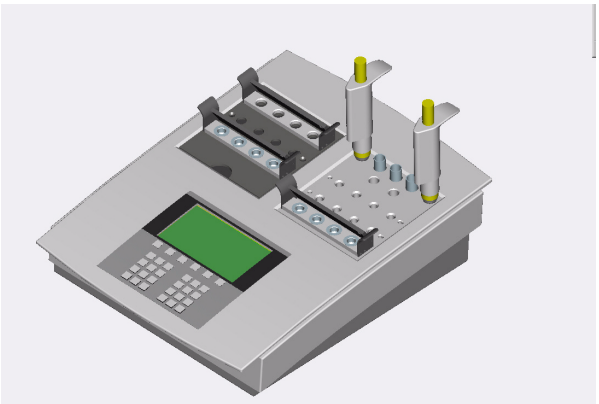
SIGMA AMELUNG

KC 4

COAGULATION ANALYZER



OPERATION MANUAL

Instrument manufactured by
Sigma Amelung,
Lemgo, Germany

REVISION DATE 10/23/01

SIGMA DIAGNOSTICS INSTRUMENT WARRANTY

Sigma-Aldrich Co., Inc. ("Sigma"), warrants that instruments it sells to be free from defects in workmanship and materials during normal use by the original purchaser.

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KC4 Δ™ User Manual, October 2001

(Software Version 1.1B)

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KC4 Δ™

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Introduction

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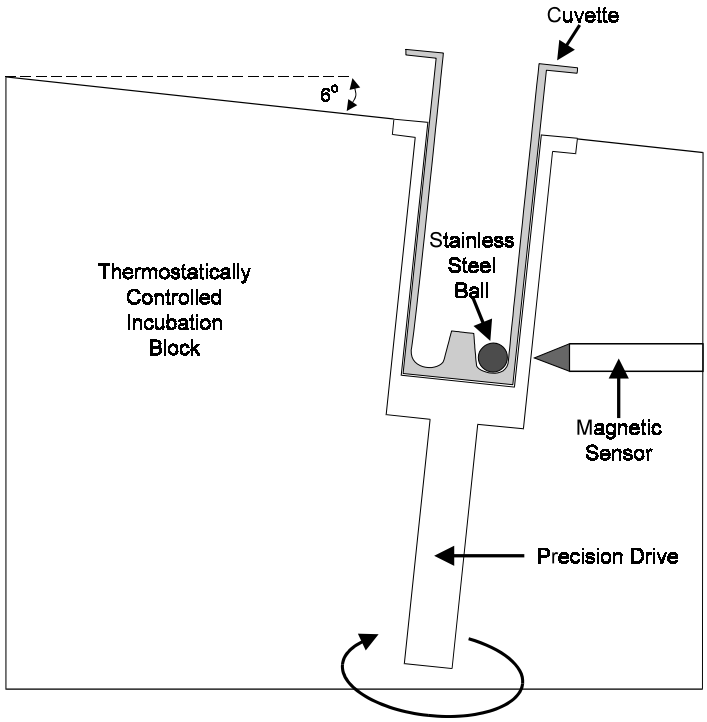
1.1 Intended Use

The KC4 Δ™ Coagulation Analyzer is a semi-automated mechanical clot detection system designed for the determination of prothrombin times (PT), activated partial thromboplastin times (APTT), fibrinogen concentrations determined by Clauss methodology, and other clotting assays. Any clotting based assay, which has fibrin formation as its endpoint may be performed on the KC4 Δ™ Coagulation Analyzer. Measurement can be qualitative or quantitative. When used in conjunction with appropriate reagents, the sample can be plasma, or whole blood.

The additions of both sample and reagents are manual. Time measurement of the clotting endpoint is automated.

1.2 Principles of Operation

The KC4 Δ™ is an electromechanical clot detection system. The system utilizes a special cuvette in which there is a stainless steel ball. Sample is added to the cuvette. After an appropriate incubation period, the cuvette is placed into the measuring well of the KC4 Δ™. The measuring well rotates slowly (50 rpm) causing the cuvette to rotate along its longitudinal axis. Because the cuvette is positioned at a slight angle, gravity and inertia always position the ball at the lowest point of the cuvette. Exactly opposite the ball-position is a magnetic sensor. With the addition of appropriate reagent, a timer is started. As coagulation takes place, fibrin strands form in the reaction mixture. The fibrin strands pull the ball away from its position and triggers an impulse in the magnetic sensor. This impulse electronically stops the timer (see diagrams).



1.3 Physical Specifications

Type:	Coagulation Analyzer, Bench Top
Online:	Unidirectional
Principle:	Ball Method
Measuring Channels:	4
Display:	LCD
Incubation Wells:	8
Reagent Wells:	5
Dimensions	
Height:	12.0 cm
Width:	35.4 cm
Depth:	45.0 cm
Weight	6.3 kg
Power Supply	
Voltage	110–220V/50–60 HZ
Power Consumption	1.5A at 100V; 0.4A at 220V
Temperature Control	
Reagent Warming Wells:	37.0°C ± 0.5°C
Reaction Incubation Wells:	37.0°C ± 0.5°C
Measurement Wells:	37.0°C ± 0.5°C
Measurement Time	
Minimum:	4.5 seconds
Maximum:	999.9 seconds

1.4 Performance Specifications

The overall performance of any testing performed on the KC4 Δ™ Coagulation Analyzer is dependent not only on the instrument performance, but is also a function of specimen integrity (collection and handling) as well as accuracy and precision of the sample and reagent dispensing system being used.

Correlation:
The following linear regression data was obtained during evaluation to show equivalence with a commercially available mechanical coagulation analyzer.

	Prothrombin Time	Activated Partial Thromboplastin Time
Number of Samples	121	110
Correlation Coefficient (r)	0.998	0.896
Slope	1.051	1.235
Intercept	–0.241	0.873

The following linear regression data was obtained during evaluation to show equivalence with a commercially available photo-optical coagulation analyzer.

	Fibrinogen	Factor X	Factor IX
Number of Samples	109	112	101
Correlation Coefficient (r)	0.930	0.974	0.897
Slope	1.067	1.010	0.958
Intercept	30.749	–0.166	3.403

The following linear regression data was obtained in three physician's office laboratories (POL) during evaluation to show equivalence with manufacturer derived results on the KC4 Δ™ Coagulation Analyzer.

POL #1	Prothrombin Time	Activated Partial Thromboplastin Time
Number of Samples	47	44
Correlation Coefficient (r)	0.991	0.960
Slope	0.981	1.066
Intercept	0.492	0.379

POL #2	Prothrombin Time	Activated Partial Thromboplastin Time
Number of Samples	45	46
Correlation Coefficient (r)	0.989	0.965
Slope	1.019	1.029
Intercept	–0.248	1.021

POL #3	Prothrombin Time	Activated Partial Thromboplastin Time
Number of Samples	52	47
Correlation Coefficient (r)	0.974	0.927
Slope	1.012	0.786
Intercept	0.326	9.470

Precision: Prothrombin Time (PT)

Imprecision was evaluated at three levels according to the NCCLS EP5-T2 protocol.

	Low	Mid	High
Mean (seconds)	13.20	33.53	39.66
Total Imprecision (CV%)	2.03	2.50	4.18
Within-Run Imprecision (CV%)	1.02	1.28	1.53

PT total imprecision was evaluated in three physician's office laboratories (POL) at three levels according to NCCLS EP10-T protocol. Within-Run imprecision was evaluated in three physician's office laboratories at two levels.

POL #1	Low	Mid	High
Mean (seconds)	13.1	26.8	42.9
Total Imprecision (CV%)	1.97	1.63	2.47
Mean (seconds)	12.7		44.1
Within-Run Imprecision (CV%)	1.3		1.1

POL #2	Low	Mid	High
Mean (seconds)	12.1	23.3	40.7
Total Imprecision (CV%)	2.59	7.12	3.0
Mean (seconds)	12.1		41.7
Within-Run Imprecision (CV%)	2.6		1.8

POL #3	Low	Mid	High
Mean (seconds)	11.3	22.8	34.2
Total Imprecision (CV%)	1.57	7.41	0.50
Mean (seconds)	11.4		34.7
Within-Run Imprecision (CV%)	2.0		1.3

Precision: Activated Partial Thromboplastin Time (APTT)
Imprecision was evaluated at three levels according to the EP5-T2 protocol.

	Low	Mid	High
Mean (seconds)	28.55	51.01	75.78
Total Imprecision (CV%)	3.12	3.41	3.21
Within-Run Imprecision (CV%)	1.47	1.60	1.37

APTT total imprecision was evaluated in three physician's office laboratories (POL) at three levels according to NCCLS EP10-T protocol. Within-Run imprecision was evaluated in three physician's office laboratories at two levels.

POL #1	**Low**	**Mid**	**High**
Mean (seconds)	29.0	43.3	57.6
Total Imprecision (CV%)	2.83	3.15	1.87
Mean (seconds)	30.8		57.5
Within-Run Imprecision (CV%)	2.7		1.6

POL #2	**Low**	**Mid**	**High**
Mean (seconds)	29.2	42.7	57.0
Total Imprecision (CV%)	4.38	2.29	2.84
Mean (seconds)	28.2		57.1
Within-Run Imprecision (CV%)	2.1		1.7

POL #3	**Low**	**Mid**	**High**
Mean (seconds)	30.0	54.7	68.6
Total Imprecision (CV%)	1.87	1.80	2.13
Mean (seconds)	26.9		64.4
Within-Run Imprecision (CV%)	1.4		2.5

Precision: Fibrinogen
Imprecision was evaluated at three levels according to the NCCLS EP5-T2 protocol.

	Low	Mid	High
Mean (mg/dl)	104.09	154.10	323.53
Total Imprecision (CV%)	3.53	6.21	4.36
Within-Run Imprecision (CV%)	2.05	2.86	2.12

Precision: Factor X
Imprecision was evaluated at three levels according to the NCCLS EP5-T2 protocol.

	Low	Mid	High
Mean (%)	31	57	102
Total Imprecision (CV%)	8.28	5.71	5.22
Within-Run Imprecision (CV%)	2.63	2.22	2.20

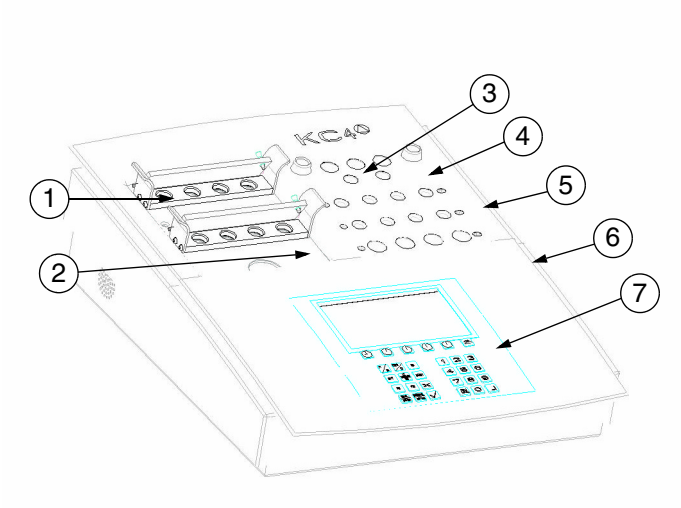
Precision: Factor IX
Imprecision was evaluated at three levels according to the NCCLS EP5-T2 protocol.

	Low	Mid	High
Mean (%)	24	49	98
Total Imprecision (CV%)	5.88	6.89	4.06
Within-Run Imprecision (CV%)	3.96	4.04	2.54

1.5 Physical Description

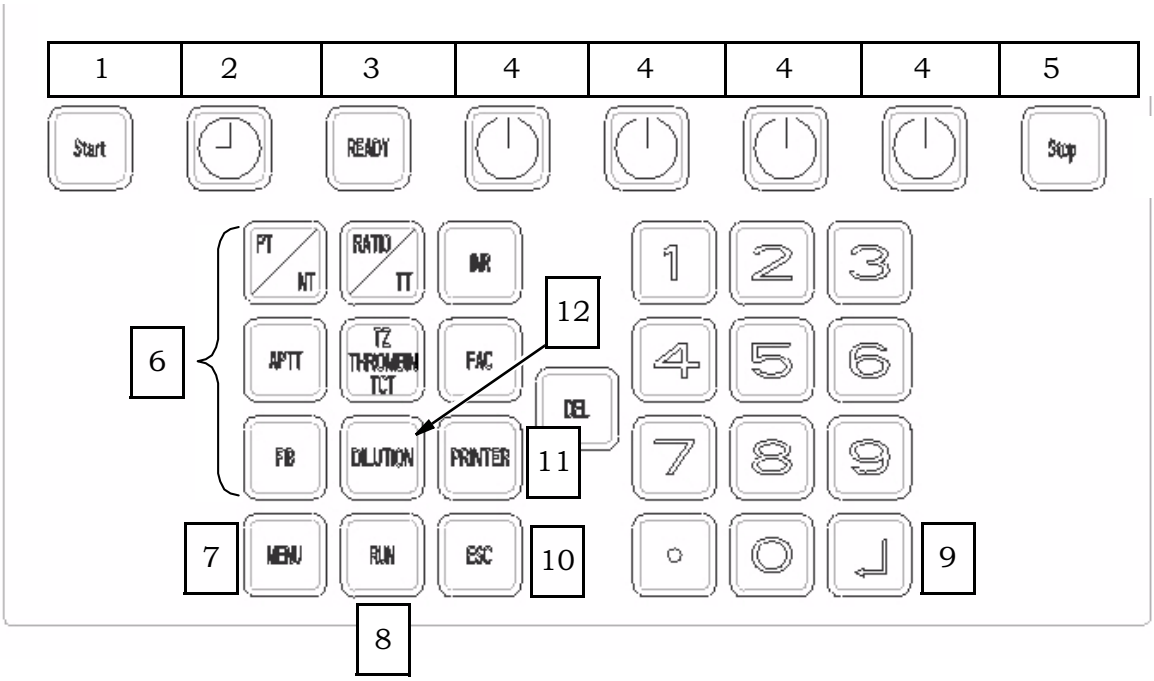
The external features of the KC4 Δ™ Coagulation Analyzer are shown in **Figure 1** (Front), **Figure 2** (Keypad), **Figure 3** (Rear), **Figure 4** (Automatic Multipette with starter cable), **Figure 5** (Ball Dispenser) and **Figure 6** (Thermal Printer).

1.6 Front View (Figure 1)



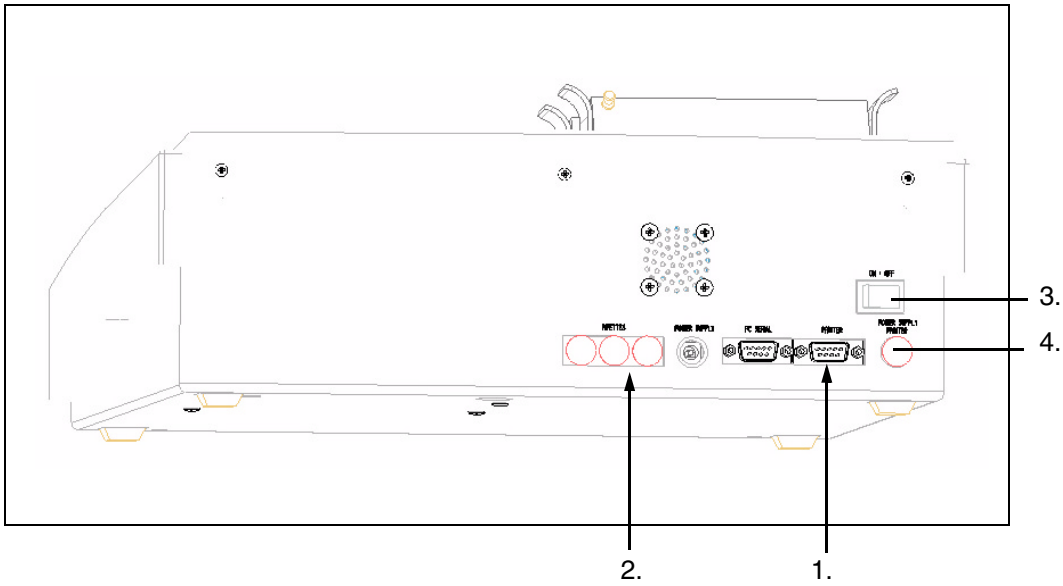
Item	Function or Description
1. Rack	Used for transferring cuvettes from preparation area to reaction incubation wells and rotating test positions.
2. Preparation Area	Room temperature wells used for sample preparation prior to incubation.
3. Pipette Tubes	Used to store the pipettes when not in use.
4. Reagent Warming Wells (5)	Three 15 mm, and two 11 mm heated wells used to warm reagents.
5. Reaction Incubation Wells (8)	Heated wells used for incubation of sample and first reagent.
6. Rotating Test Positions (4)	Positions where start reagent is added and the clotting time is measured.
7. Display Screen	Displays elapsed time in seconds during incubation for each of 4 channels. Displays elapsed time in seconds and tenths of seconds during clot time measurement. Displays incubation times, clotting times, programming selections and other menus.

1.7 Keypad (Figure 2)



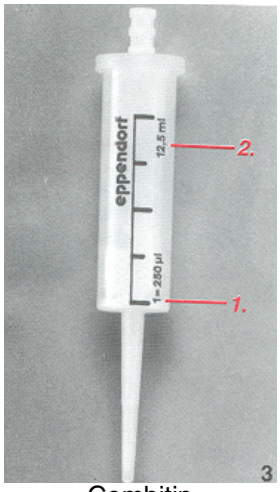
Item	Function or Description
1. **Start** Key	Activates automatic measurement timer.
2. **Incubation** Key	Starts Incubation timers.
3. **Ready** Key	Not functional at this time.
4. **Channel** Key	Starts manual timing and incubation.
5. **Stop** Key	Terminates measurements, and aborts testing.
6. Function Keys	Used in programming tests.
7. **Menu** Key	Returns to **Main** Menu from **Operating** Screen.
8. **Run** Key	Returns to **Test Program Selection Screen** from **Operating Screen**.
9. ↵	**ENTER**
10. **ESC** Key	Escapes back to previous function
11. **Printer** Key	Used to turn printer on or off
12. **Dilution** Key	Used to change the patient dilution.
13. **DEL** key	

1.8 Back View (Figure 3)

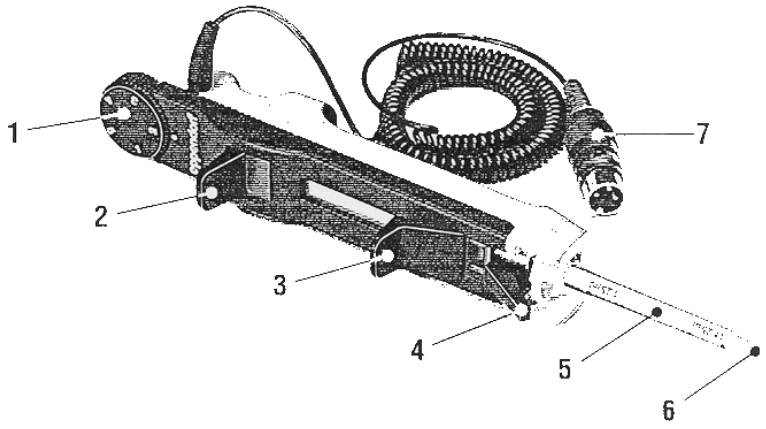


Item	Function or Description
1. Thermal Printer Port	Thermal Printer connection.
2. Automatic Multipette Socket(s)	Used to connect pipettes (Remove the cap-plug in the Multipette).
3. Power Switch	Powers instrument off/on.
4. Power Supply Socket	Connects instrument to power cord.

1.9 Multipette (Figure 4 A & B)



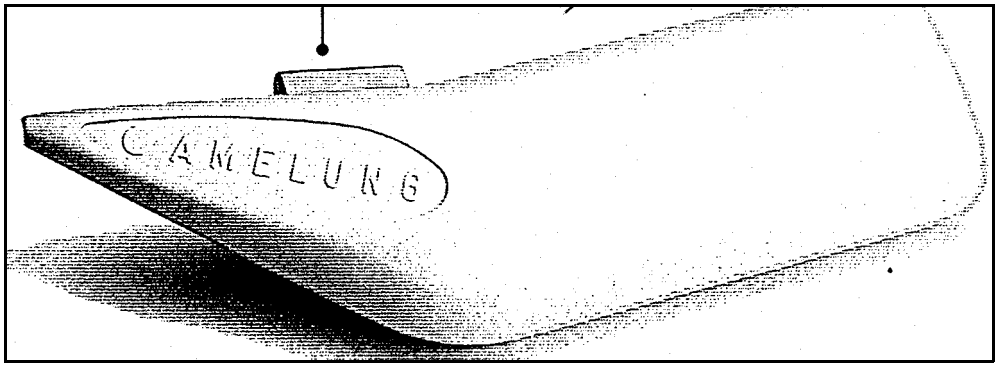
Combitip
4-A



Multipette
4-B

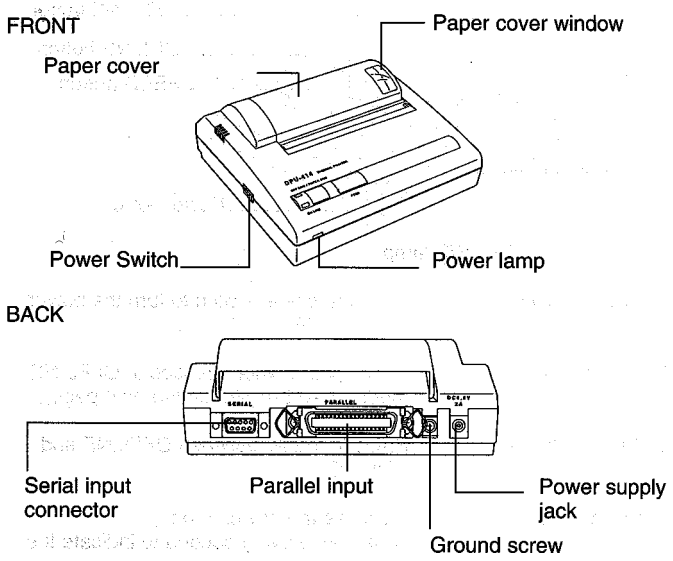
Item 4-B	Function or Description
1. Volume selection dial	Determines pipetting volume: setting (1–5) multiplied by the minimum pipetting volume of the Combitip (1.25 ml or 2.50 ml pipette tips).
2. Pipetting lever	The volume is pipetted by pressing the pipetting lever down until it stops.
3. Filling lever	The Combitip is filled by sliding this lever upward.
4. Locking clamp	The locking clamp serves to firmly clamp the Combitip.
5. Combitip	The pipette tip used with the automatic multipette.
6. Combitip Cone	Portion of the pipette tip that aspirates reagent.
7. Starter Cable	Connects pipette to instrument.

1.10 Ball Dispenser (Figure 5)



Item	Description or Function
1. Dispenser	For loading microballs into cuvettes

1.11 Thermal Printer (Figure 6)



1.12 Printer Options

From the **Main Menu**, press **<↵>**. Press the <**PRINTER**> key. The (*) shows which options have been selected. If the analyzer is connected to a host LIS, ensure that option **4** is selected. If the analyzer is not connected to a host LIS, select **5**.

Print Test Page	1
Print Switch On	2*
Print Switch Off	3
Line - out incl. Error Val.	4*
Line - out excl. Error Val.	5
Press ENTER <↵> to continue	

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2.1 Unpacking

The **KC4 Δ™** Coagulation Analyzer is shipped in a transport box designed to protect the instrument from damage during shipment. If damage is apparent, immediately notify the shipping company. Note the damage on the shipping bill of lading and notify your Sigma Diagnostics Sales Representative.

2.2 KC4 Δ™ Coagulation Analyzer Start-Up Kit

Carefully remove the instrument and accessories from the transport box. Check that the following items have been included:

KC4 Δ™ Coagulation Analyzer

1. Power Cable
2. KC Micro Tetravettes
3. KC Delta Multipette with Starter Cable and Adapter Cord
4. Combitips (1.25 ml); 5 each
5. KC Pipette Tips, Yellow 200µl; 1 tray
6. Tubes, Plastic (14.5 x 85 mm); 100 each
7. Tubes, Glass (15 x 85 mm), 50 each
8. Power Supply 12V
9. Lead for Power Supply
10. Protective Dust Cover; 1 each

Optional Items

	Catalog Number	Item
1.	P2864	KC Series Printer with Power Adapter
2.	K1638 *	KC Series Thermal Printer Paper
3.	K4882	KC4 Δ™ Multipette with Starter Cable
4.	K0508 *	KC4 Δ™ Combitips 1.25 ml
5.	K1510 *	KC4 Δ™ Combitips 2.50 ml
6.	K4257 *	KC Pipette Tips, Yellow, 200µl, 10 trays
7.	T9304 *	Tubes, Glass (15 x 85 mm), 50 each
8.	K4887 *	KC4 Δ™ Tetravettes
9.	T2242*	Tubes, plastic (14.5 x 85 mm)
10.	K1635*	KC Micro Cuvettes with Ball Dispencer
11.	A6083	Coated Stir Bar
12.	K6208	APTT Stir Bar
13.	K0633	KC Pittette Tube Sleeves

***These are consumable items and should be ordered as needed.**

Pipettes are required for the test performance. Although the use of a Multipette will ensure the start of the timing measurement is simultaneous with the addition of the reagent, it is not mandatory.

Read the Operation Manual carefully prior to using the KC4 Δ™ Coagulation Analyzer. The Operation Manual has been written to provide the most comprehensive understanding of the operation of the KC4 Δ™ Coagulation Analyzer and to enable you to fully utilize the features of the instrument.

2.3 Location Requirements

1. Place the KC4 Δ™ Coagulation Analyzer on a stable, vibration and dust free work surface. It should not be positioned next to a centrifuge or other equipment, which may cause vibration. The KC4 Δ™ Coagulation Analyzer should also be protected from moisture.
2. To avoid exceeding the control range of the instrument, place the KC4 Δ™ Coagulation Analyzer in an area with a maximum room temperature of 30°C. It should not be positioned in an area directly below ventilating ducts which produce strong air currents. Do not expose the KC4 Δ™ Coagulation Analyzer to direct sunlight. Sunlight influences the temperature control.
3. It is preferable to place the KC4 Δ™ Coagulation Analyzer in an area which is no further than (6 ft.) 1.8 m from an electrical outlet. The instrument should not be operated from an extension cord which does not employ protective grounding. The electrical outlet used should not be shared with any devices, which consume large amounts of power on a cyclic basis (e.g., centrifuges, air conditioners, and refrigerators). When these type of devices cycle on and off, there may be a voltage drop in the line which could interfere with the proper functioning of the instrument.

2.4 Electrical Requirements

The KC4 Δ™ Coagulation Analyzer is designed with a factory equipped three-pronged grounding plug designed to be connected to the Power Supply, which is then plugged into the analyzer. Under no circumstances should it be connected to an ungrounded two-pronged receptacle. This procedure is in accordance with the National Electrical Code and other applicable ordinances for this type of installation.

1. Do not use an extension cord which cannot to provide protective grounding.
2. It is recommended that any repair work other than routine maintenance be performed by a trained specialist familiar with the hazards involved.
3. If safe operation of the KC4 Δ™ Coagulation Analyzer is no longer possible, the instrument must be taken out of service.

2.5 Preliminary Check of the Instrument Operation

The preliminary function checks of instrument operation should be performed prior to using the instrument. This preliminary function check is to ensure that the instrument is functioning properly prior to reporting patient results.

1. Connect the power cable to the power cable socket on the back of the instrument (DC6.5V 2A).
2. Connect the Data Cable from the Serial Port on the Printer to the Printer port on the back of the Analyzer if utilizing the KC4 Δ™ printer.
3. Activate the KC4 Δ™ Coagulation Analyzer by pressing the off/on switch located on the left hand side of the back of the instrument.
4. Observe that the display screen lights up; a screen appears giving the operator the option to select the operating language. After the language selection, a screen showing a thermometer appears and will remain displayed while the instrument warms up to 37°C.
5. Observe that four of the measurement wells are rotating. The wells will rotate continuously whenever the instrument is on.
6. Place a KC4 Δ™ Micro cuvette or Tetravette into each position of the cuvette rack. Place the cuvette rack on the rotating test positions such that the cuvettes are sitting flush in the holes. If using a KC4 Δ™ Micro cuvette, dispense one ball into each cuvette using the ball dispenser. Observe that the ball falls to the front of the cuvette and stays there.
7. Verification of temperature can be performed by placing approximately 3 ml of water into a 15-mm reagent tube. Place a thermometer into the tube and allow to equilibrate until the temperature has stabilized. Approximately 15 minutes will be required for temperature stabilization. The temperature should be 37° ± 0.5°C.

Note: The use of smaller diameter tubes is not recommended due to inadequate heat transfer.

8. To verify the operation of the timers, use the pipette with the start cable, and the measuring wells, a program modification is needed. From the **Main Menu,** select **3 Program Selection**.
9. Enter the password; default password is **1 2 3 4**. Press <↵>.
10. Select **4 Individual Program Modify**; press **TZ (Thrombin Clotting Time)** key. Press <↵>.
11. To access the program settings, press <↵>. Press **2 (No)** until "Test TZ" appears at the top of the screen. Press **1 (Yes)** to modify TZ.
12. Enter **1 (duplicate testing)**, and 10% for allowed CV; press <↵> to continue.
13. Enter incubation time of **10 seconds**, press <↵>. Press **2 (No)** when modifications are done.
14. Press <↵> to continue. Press **Run**; select **3 Start Individual Program**. Press **TZ (Thrombin Clotting Time)** key; press <↵>.

15. Enter 2 samples per rack; press <↵>. Press <↵> again to bring the **Operating Screen** up.
16. If the automatic Multipette with the starter cable is being used, plug the pipette cable connection into the pipette cable socket on the rear of the KC4 Δ™. Snap the locking mechanism on the Multipette into place.
17. Start timers by pressing the **<START>** key, followed by the individual well timers . When all timers are showing 0.0**, press and hold** the **<START>** key , while at the same time, depress the trigger switch on the Multipette 4 times. All wells should begin timing.
18. After at least 10 seconds, remove the cuvette rack from the rotating test positions. Observe that the timers stop and are indicating the elapsed time in seconds and tenths of seconds.
19. The first result will print automatically (if the optional printer has been installed), or will appear on the screen. Press <↵> to print the second result, and clear the memory.

With the completion of the Preliminary Checks of Instrument Operation, installation is complete and the instrument is ready for operation. If the instrument fails to perform any of the tests with the specifications listed, call Sigma Diagnostics Technical Service for assistance.

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3.1 Instrument Preparation

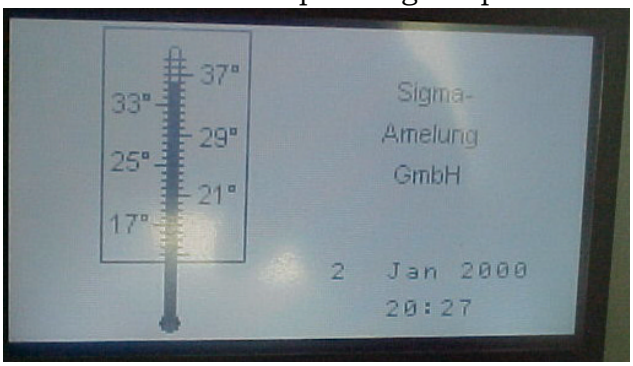
The KC4 Δ™ is activated by the on/off button located on the back of the instrument. Turn instrument on and select appropriate language. Press <↵>.

Note: This screen will only appear the first time the analyzer is turned on. After a language has been selected, the analyzer will automatically display the temperature indicator screen (shown below) on each subsequent power on.

Sprachwahl.	
Deutsch	1
Englisch	*2
Franzoesisch	3
Italienisch	4
Niederlaendisch	5
Spanisch	6
Weiter mit Taste ENTER	

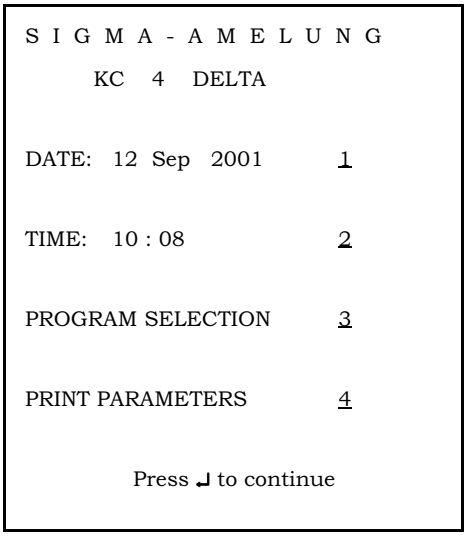
3.2 Temperature Indicator Screen

In this figure, the thermometer indicates operating temperature has not been reached.



In approximately 20 minutes the analyzer will reach operating temperature (37° ± 0.5°C). The display will change to the **MAIN MENU**:

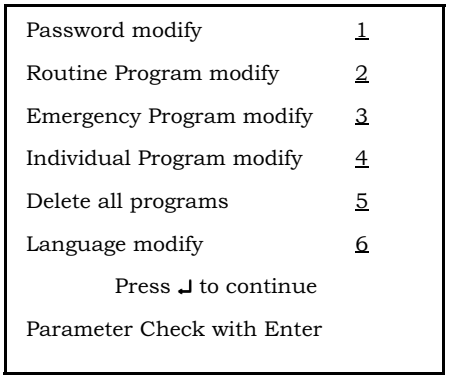
3.3 Main Menu Functions



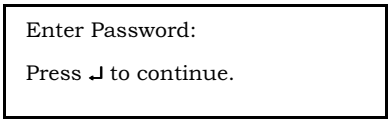
1. To enter **DATE**, select **1**. The date is in European format: dd,mm,yy. 12-09-2001 is September 12, 2001. The entry is confirmed with **<↵>** or terminated with **<ESC>**. If terminated, the previous values remain valid.
2. To enter **TIME**, select **2**. The format for time is hh:mm; example: 10:08
3. To modify **Program Selection**, select **3**.
4. To **PRINT** a copy of all programmed tests and parameters, select **4**.
5. To proceed to the **Operating Screen**, press **<↵>**.

3.4 Password Modification

From the **Main Menu** screen, press **3 Program Selection**.



Select **1**, **Password modify**. Enter a new password, **Press <↵>**. The default password for a new analyzer is **1 2 3 4**.



3.5 Program Modification

Selecting the tests to be available for each program

The programs (Routine, Emergency, Individual) are modified by selecting tests from the keypad to form a desired group.

From the **Main Menu** screen, select **3 Program Selection**. From the **Program Selection** screen, select:

2 Modify the **Routine Program**: This function allows the operator to select the tests available to be run in the **Routine Program**. The tests are selected by pressing keys (**INR**, **APTT**, **FIB**, etc.) and confirming with **<↵>**. Terminating is possible with **<ESC>**. Upon termination, the previously set values will remain valid. In this program, the analyzer will allow a sequential operation of tests (**Batch – mode**).

3 Modify the **Emergency Program**: This function allows the operator to select the tests available to be run in the **Emergency Program**. The tests are selected by pressing keys (**INR**, **APTT**, **FIB**, etc.) and confirming with **<↵>**. Terminating is possible with **<ESC>**. Upon termination, the previously set values will remain valid. The analyzer will do a selected profile of tests on a single patient (**Patient – Selective**).

4 Modify the **Individual program**: This function allows the operator to individualize the test menu by sample if desired. The tests in the **Individual Program** are chosen directly from the keyboard by pressing keys (**INR**, **APTT**, **FIB**, etc) and confirming with **<↵>**. Terminating is possible with **<ESC>**. Upon termination, the previously set values will remain valid.

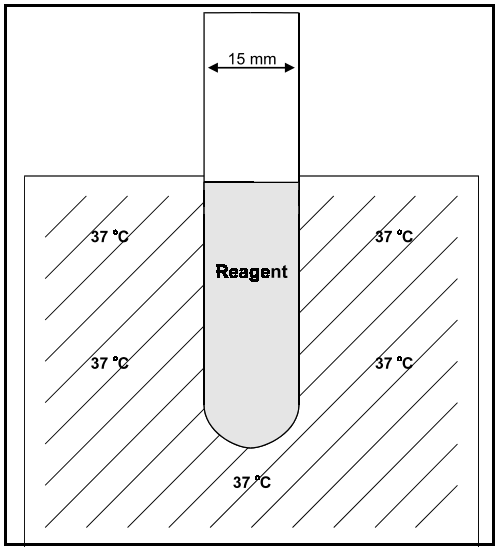
5 To delete all programs.

6 Language Modify
Parameter Check with Enter

WARNING: All tests in the programs and all test parameters can be deleted with one keystroke. A complete new installation of programs is necessary if all programs are deleted.

See **Section 4** for a more detailed description of Program Modification.

3.6 Reagent Handling



Reagents for the appropriate test are prepared according to the manufacturer's instructions. Refer to the manufacturer's reagent application for specific instructions on preparation and handling of reagents. Any reagent requiring pre-heating should be placed into a 15-mm tube and inserted into the reagent incubation well. The fluid level in the tube should not be above the top edge of the incubation well. A minimum of 15 minutes will be required to warm reagent to 37°C ± 0.5°C. **(Note: Reagents direct from the refrigerator may take slightly longer to warm up.)** All reagents should be used before the expiration date listed by the manufacturer for each reagent. Do not place any opened reagent bottles on the instrument.

3.7 Cuvette Preparation

Tetravettes or micro cuvettes are placed in racks in the unheated preparation area. Up to twelve (12) cuvettes can be placed on the instrument. The exact size and surface quality of the cuvettes is critical to the proper performance of testing. Absolute cleanliness of cuvettes is mandatory to ensure correct performance. The cuvettes are intended as one-time use items. Rewashing cuvettes is not recommended. The performance of substitute cuvettes cannot be guaranteed; therefore, substitute cuvettes should not be used.

Tetravettes are cuvettes in pre-packaged groups of 4. They contain a stainless steel ball and can go directly into a loading rack and onto the instrument.

The stainless steel balls are manufactured from a special stainless steel. Purity, weight, size, surface quality and magnetic characteristics of the balls are all critical to the proper performance of testing. The balls, which are manufactured by Sigma/Amelung have been tested to ensure compatibility with the instrument measurement process and that they are inert when used with plasma and coagulation reagents. Rust, slight impurities or oil residue can have an adverse effect on coagulation testing results. The balls are intended as one-time use items. Recycling balls is not recommended. The performance of substitute balls cannot be guaranteed; therefore, substitute balls should not be used. The ball dispenser used in conjunction with the micro cuvettes, has been designed to facilitate transfer of balls into the cuvettes.

3.8 Sample Preparation

Plasma samples and reagents are added with appropriate microliter pipettes. Refer to the manufacturer's reagent application to determine the sample and reagent(s) volume required for each test. Pipetting technique is critical to the performance of testing. Refer to the Pipetting section for guidelines in proper pipetting technique. Although the use of a Multipette will facilitate initiation of measurement timing, special pipettes are not required. If a Multipette is not available, measurement timing can be started using the start button and channel buttons.

Sample is dispensed into the cuvette. Twelve o'clock is the recommended dispense position. After sample has been dispensed, close the Plexiglas flap, pick the cuvette rack up and swirl gently to evenly disperse the sample over the bottom of the cuvettes. Place the rack with cuvettes in the rotating test positions ensuring that the cuvettes are pressed firmly down to the bottom. Two additional racks of cuvettes (4 each) may also have samples pipetted and placed in the reaction incubation wells.

Care must be taken not to over incubate the samples. It is advisable to stagger the time interval between the cuvette racks in the heated incubation wells and rotating test positions to prevent over incubation. Allow the samples to pre-incubate for the recommended time. It will take slightly longer (a minimum of 120 seconds) for a sample that has been stored at refrigerator temperature (2–8°C) to reach 37°C than for samples stored at room temperature (18–26°C). Several of the coagulation factors, (Factors V, VIII, XIII, and fibrinogen) are labile at 37°C. To avoid loss of these factors, samples should not be pre-incubated longer than 5 minutes.

Timing is critical in coagulation testing and the reagent manufacturer's guidelines for incubation times should be followed. Any particulate reagent must be well mixed prior to use. For those tests, having more than one reagent, all incubations prior to measurement start can be accomplished in the reaction incubation wells. Nine o'clock is the recommended first-reagent dispense position.

Care must be taken to avoid contact of the reagent pipette tip with previously dispensed sample. After addition of reagent, close the Plexiglas lid, pick up the cuvette rack, and swirl gently 5–6 times to mix the reagent and previously pipetted sample. The reaction mixture should be evenly dispersed around the channel at the bottom of the cuvette. No more cuvettes should be prepared for testing than can be completed within the specified guidelines.

In most instances, addition of a start reagent begins the coagulation process. It is important that the **Measurement Timer** or Multipette be started simultaneously to the addition of the start reagent in order to ensure accuracy and precision of the assay. Test measurement timing can be started either manually using the **Channel Key** or automatically by using the Multipette fitted with a starter cable. Use of a Multipette will ensure that the reagent addition starts measurement timing. Directions for both manual start and automatic start are described below.

The location of the start reagent dispense is important. To ensure that mixing of start reagent with the previously pipetted sample or sample/reagent mixture begins immediately, the start reagent should be dispensed just to the right side of the ball. This is best accomplished by holding the pipette angled obliquely from the right rear side of the cuvette towards the ball position and dispensing the reagent just to the right

of the ball. Care must be taken to avoid splashing the reagent out of the cuvette. The dispense rate should be of moderate speed and forcefulness.

3.9 Pipetting

The overall performance of the KC4 Δ™ Coagulation Analyzer is dependent on the accuracy and precision of pipetting both sample and reagent(s).

Testing can be performed with either standard microliter pipette(s) or with the Multipette fitted with a starting cable. When the Multipette is used to dispense the final start reagent, the timer is automatically started simultaneously with reagent dispense. When a standard microliter pipette is used for addition of the final start reagent, the timer is started manually using the **Specific Channel Key**.

Regardless of what kind of pipette is used, the care taken with pipetting is directly proportional to the overall accuracy and precision of testing.

To avoid contamination of reagents, if the same pipette is being used for both sample and reagent, a new tip must be used when transitioning between sample and reagent.

To avoid cross-contamination between samples, a new tip should be used for each sample, whether running plasma or whole blood samples.

Pipetting Technique for Non-Repeating Pipettes

To fill the pipette tip: Depress the button to the first stop. With the button depressed, insert the tip into the sample or reagent to a depth of approximately 2–3 mm. If pipetting plasma directly from a centrifuged tube of blood, the tip should be kept well away from the blood/plasma interface. This will assure that no red cells or platelets will be aspirated into the tip. If pipetting a particulate reagent, the reagent should be well mixed prior to aspiration.

Release the button slowly in such a manner that the sample or reagent flows smoothly into the pipette tip. Slow aspiration will assure that the volume aspirated into the tip is accurate. If the button is allowed to snap back, an incorrect volume may be aspirated. In addition, sample or reagent can be aspirated into the barrel of the pipette. This can result in contamination of subsequent samples or reagents. Unless the pipette is dismantled and cleaned, inadvertent aspiration into the pipette barrel will result in eventual obstruction and incorrect operation of the pipette.

Once the tip is filled, no dripping should be observed. If dripping is observed, either the tip is not seated correctly on the pipette or the pipette requires maintenance. In such a circumstance, replace the tip. If this does not correct the problem, the pipette should not be used until maintenance can be performed.

Pipetting Technique for Multipettes

Only pipette tips recommended for use with the pipette should be used. Any pipette tip whose insertion opening is out-of-round should be discarded. Any pipette tips that are bent or otherwise damaged should be discarded. The tip opening must not be occluded.

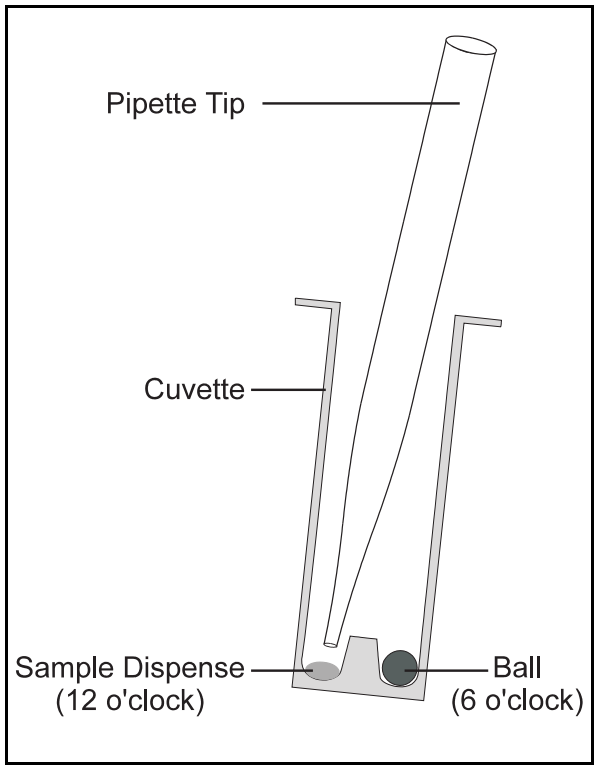
Slide the filling lever down until it stops, then raise the locking clamp upward.

Insert the combitip until it clicks into position. Be sure the combitip plunger is fully inserted into the barrel before attaching it to the Multipette. Be sure the filling lever is completely down and then lower the locking clamp to secure the combitip in place.

Verify that the volume selection dial is set to dispense the correct volume. Immerse the combitip cone into the liquid. Fill by slowly sliding the filling lever upward. Wipe the combitip cone with a lint-free tissue. Follow the guidelines for correct dispense positions described in the following sections.

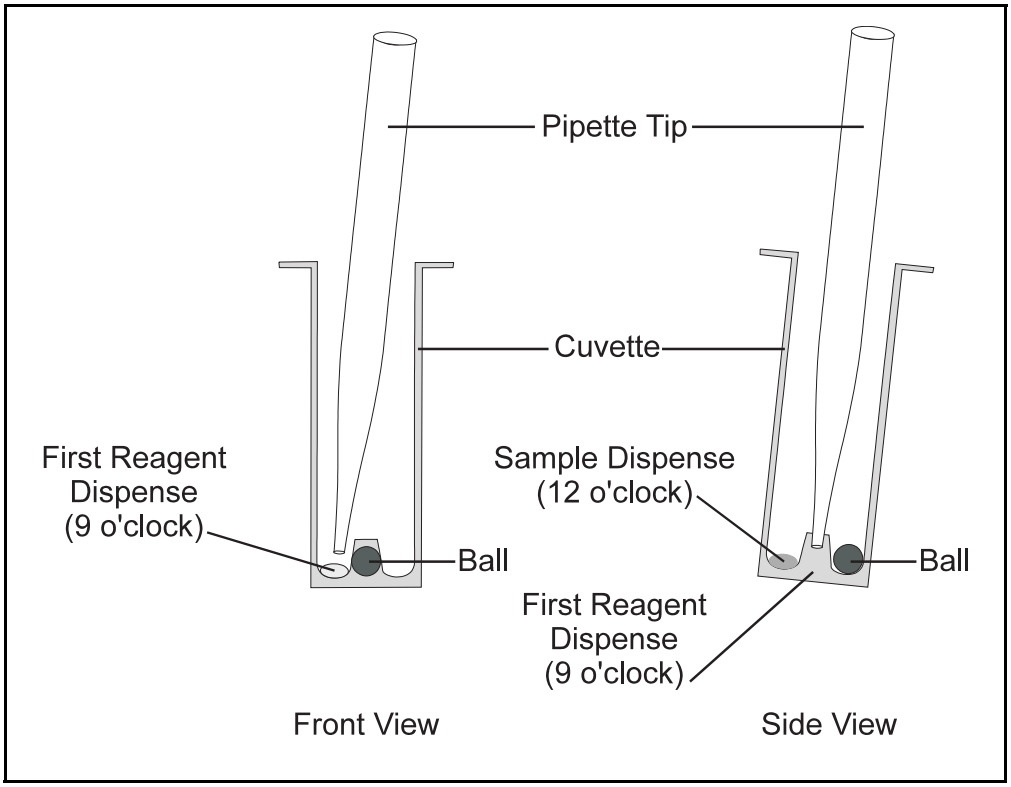
3.10 To Dispense Sample

Sample should be dispensed to the 12 o'clock position of the cuvette (see diagram). Aim the pipette to the 12 o'clock position. Position the tip approximately 3–4 mm above the bottom of the cuvette. Depress the pipette button to the first stop and hold for 1–2 seconds to allow the residual contents of the tip to collect at the bottom of the tip. Press the button to the second stop. This will deliver any residual sample into the cuvette. To avoid bubbling and splattering, the tip should not be placed so close to the bottom that at the completion of pipetting the tip is submersed in the dispensed sample. An alternative method is to touch the tip to the cuvette sidewall approximately 3–4 mm above the bottom of the cuvette and then depress the button slowly to the first stop. Wait 1–2 seconds and press the button to the second stop position. Sample should not be dispensed by touching the tip to the upper sidewalls of the cuvette. Any sample that is left on the upper walls will not participate in the coagulation reaction.



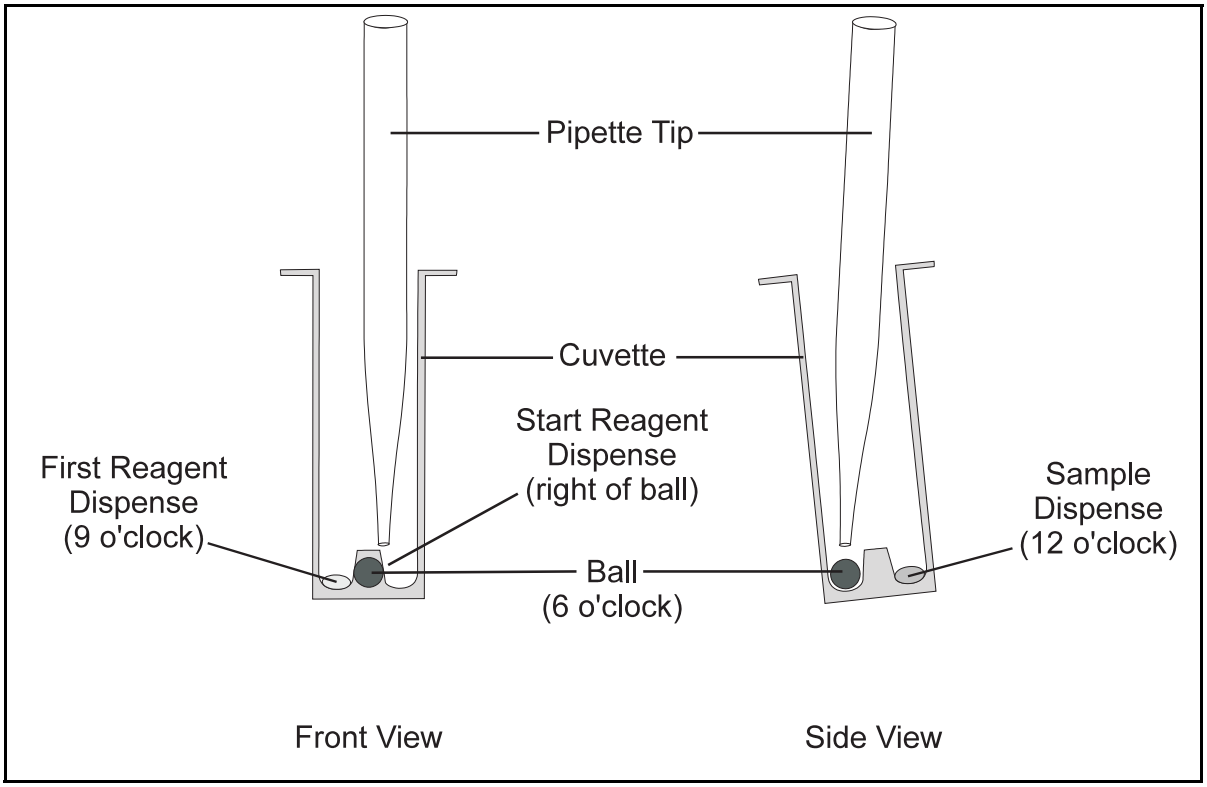
3.11 To Dispense First Reagent

For those tests utilizing more than one reagent, the first reagent should be dispensed to the 9 o'clock position of the cuvette (see diagram). Aim the pipette to the 9 o'clock position. Position the tip approximately 3–4 mm above the bottom of the cuvette. Depress the pipette button to the first stop and hold for 1–2 seconds to allow the residual contents of the tip to collect at the bottom of the tip. Press the button to the second stop. This will deliver any residual reagent into the cuvette. To avoid bubbling and splattering, the tip should not be placed so close to the bottom that at the completion of pipetting the tip is submersed in the dispensed reagent. An alternative method is to touch the tip to the cuvette sidewall approximately 3–4 mm above the bottom of the cuvette and then depress the button slowly to the first stop. Wait 1–2 seconds and press the button to the second stop position. To avoid contamination of reagent during subsequent reagent pipetting, care must be taken to avoid contact of the tip with the previously dispensed sample.



3.12 To Dispense Start Reagent

The Start Reagent is the reagent that, when added, begins the coagulation reaction. Start Reagent should be dispensed just to the right side of the ball. This positioning assures that mixing of reagent with the other reaction constituents begins immediately. Holding the pipette obliquely from the right side, aim the pipette tip to the right side of the ball. Position the tip approximately 5–6 mm above the ball. Depress the pipette button to the last stop position. The dispense rate should not be so rapid or forceful that reagent is splashed out of the cuvette. To avoid contamination of reagent during subsequent reagent pipetting, care must be taken to avoid contact of the tip with the previously dispensed sample and/or reagent (see diagram).



3.13 Selecting a Test to Begin a Run

From the **Main Menu,** Press < ↵ >. The **Operating Screen** will be displayed.

Operating Screen

Pat. ID: 333				37°C
Position	1	2	3	4
Inc. Time	0	0	0	0
Meas. Time	0	0	0	0
Start Inc.				
Ready				
	1	2	3	4

The upper left shows the current patient ID and the upper right shows the temperature of the measuring block in °C.

<MENU> Accesses up **Main Menu** functions.

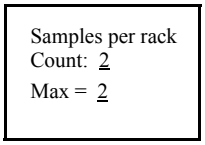
<RUN> accesses the **Program Menu**.

Select **1 Routine Program**. Select number of samples per rack, press < ↵ >. Enter the Patient ID, press < ↵>. The analyzer will display the first test programmed into the **Routine Program** (see Section 4). For example, if INR, APTT and FIB are in the **Routine Program**, then the first test to be performed will be INR. INR will appear in the upper right corner of the **Operating Screen** (replacing the temperature). To scroll to the next test in the program (ie. APTT), press < ↵>. Enter the number of samples per rack, press < ↵>. The next test in the program (APTT), will appear in the upper right corner of the **Operating Screen**. To end the program, press **<RUN>**.

Select **2 Emergency Program**. Enter Patient ID, press < ↵>. The first test programmed in the **Emergency Program** will appear in the upper right corner of the **Operating Screen**. Only one sample at a time can be run in the **Emergency Program**. At the end of the first test, the result will be displayed. Record the result, Press < ↵> to scroll to the next test in the program. To end the program, press **<RUN>**.

Select **3 Individual Program**. A grid of available tests programmed into the **Individual Program** will be displayed (see section 4). Select a test by pressing the test **Function Key**. For example, INR, APTT and FIB are displayed on the grid. To begin INR testing, press the **INR** key. Press < ↵>. Enter number of samples per rack, press < ↵>; enter Patient ID, press < ↵>. INR will be displayed in the upper right corner, and the Patient ID will be displayed in the upper left corner of the **Operating Screen**.

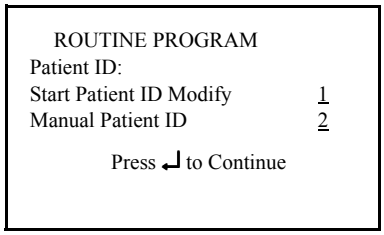
Note: When entering the number of samples per rack: if testing in duplicate, Max would be 2 and the choice of 1 pair or 2 pair per rack would be made. It is recommended that samples be analyzed in duplicate.



3.14 Patient Identification

After the number of samples per rack has been entered, the analyzer will ask for a Patient ID number. Press **1** to enter a Patient ID. The instrument will automatically increment up from this number. Press **2** to enter a specific Patient ID for the samples to be analyzed.

Note: This screen must be re-accessed to enter subsequent Patient ID numbers. To do this, press the RUN key, select 3 Individual Program. Press the appropriate test key. Theer the number of samples per rack (2 when analyzing in duplicate); select 2 for manual Patient ID entry.



3.15 Testing

1. With the transfer of the first rack to the rotating wells, Press **Incubation timer**:

Operating Screen

Patient ID: 333				INR
Position	1	2	3	4
Inc. Time	0	0	0	0
Meas. Time	0.0	0.0	0.0	0.0
Start Inc.	***	***		
READY				

2. Press **Channel timer** for each sample to be tested.

3. Each channel will begin the incubation countdown. An audible beep will occur when 5 seconds remain.
4. Press and hold down the **<START>** key and begin dispensing the start reagent.

3.16 Manual Start Procedure

Install a pipette tip onto an appropriately sized pipette.

Mix particulate reagent by covering the tube with Parafilm® and inverting gently. Do not shake. Many coagulation reagents are mixtures of lipids, which will undergo spatial rearrangement when shaken.

Aspirate reagent into the pipette tip. Dispense one aliquot of reagent into reagent container to prime the pipette tip.

Press the **<START>** key.

Dispense reagent just to the right side of the ball and simultaneously press the Specific **Channel Key** ⏻.

Note: Measurement can be started automatically if the reagent is dispensed with enough force to move the ball from its steady position. This is best accomplished by dispensing the reagent directly onto the right side of the ball. This dispense should not be so forceful that any of the reaction mixture is splashed out of the cuvette. This is not as reliable a way to start timing. It is particularly unreliable when dispensing a volume less than 100 µl.

3.17 Automatic Start Procedure

The KC4Δ™ Multipette is recommended for use in the Automatic Start Procedure.

Assure that the Multipette cable is plugged into the Automatic Pipette socket located on the rear of the KC4Δ™ Coagulation Analyzer.

Install appropriately sized pipette tip.

Mix particulate reagent by covering the tube with Parafilm® and inverting it gently. Do not shake. Many coagulation reagents are mixtures of lipids, which will undergo spatial rearrangement when shaken. The reactivity of such reagents is dependent on the specific lipid arrangement.

Aspirate start reagent into the pipette tip. Dispense one aliquot of reagent into reagent container to prime the pipette tip.

Press the **<START>** key.

Fully depress the pipetting lever to dispense reagent. This is best accomplished by holding the pipette angled obliquely from the right rear side and dispensing the reagent just to the right side of the ball. Measurement timing is started automatically.

Return the pipette to the heated pipette tube holder. **Note**: Replace the pipette tube holder sleeves daily and for each new reagent. Pipette tube holder sleeves should be treated as biohazardous waste.

Note: To increase speed when using an automatic multipette with starting cable, simple keep the Start Key of the first channel depressed while pipetting. Thus, when channel 1 is pipetted and the timer for channel 1 is started, the timer for channel 2 is activated. When channel 2 is pipetted, its timer begins and channel 3's timer is activated, etc.

With the addition of start-reagent, initiation of the coagulation process begins. As coagulation takes place, fibrinogen polymerizes to form strands of fibrin. The formed fibrin sweeps the ball out of its steady position, which triggers an impulse in the magnetic sensor. This impulse electronically stops the timer signifying the end of measurement time. The elapsed time will be displayed in seconds and tenths of seconds.

3.18 Printing Results

1. The printer will immediately print out the results of the first test. The second test results will be printed after <↵> is pressed.

Individual Program: INR
Date : 25 Nov 2001
Time : 01:11
Patient ID : 25
Measured Values:
Value 1 : 12,4 sec
Value 2 : 12,1 sec
Average Value : 12,25 sec
Difference : 2,4 %

Result : 1,02

Individual Program: INR
Date: 25 Nov 2001
Time: 01:12
Patient ID: 26
Measured Values:
Value 1 : 12,0 sec
Value 2 : 12,3 sec
Average Value : 12,15 sec
Difference : 2,4 %

Result : 1,01

Individual Program: INR
Date: 25 Nov 2001
Time: 01:14
Patient ID: 27
Measured Values:
Value 1 : 11,5 sec
Value 2 : 11,2 sec
Average Value : 11,35 sec
Difference : 2,6 %

Result : 0,93

Contents

4.1 ROUTINE PROGRAMMING 4-1

4.2 EMERGENCY PROGRAMMING 4-2

4.3 INDIVIDUAL PROGRAMMING 4-3

4.1 Routine Programming

From the **MAIN MENU**:
Select **3**, **Program Selection**.

Main Menu

S I G M A - A M E L U N G	
KC 4 DELTA	
DATE: 12 Sep 2001	1
TIME: 10 : 08	2
PROGRAM SELECTION	3
PRINT PARAMETERS	4

Enter Password, press **ENTER <↵>**.

Password Screen

Enter Password:
Press ↵ to continue.

Press **2**, **Routine Program Modify**.

Modify Screen

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Language Modify	6
Parameter Check to Enter	

Select from the keypad all tests desired for this group. When complete, press **ENTER <↵>**.

Routine Program

Tests
In Routine Program
INR APTT FIB
New entry press ENTER to store
Press ESC to cancel

Tests programmed will cycle, allowing parameter changes to be made.

See **Section 5** regarding programming tests.

Programming Screen

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Press ENTER to continue	

4.2 Emergency Programming

From the **MAIN MENU**:
Press **3**, **Program Selection**.

Main menu

S I G M A - A M E L U N G	
KC 4 DELTA	
DATE: 12 Sep 2001	1
TIME: 10 : 08	2
PROGRAM SELECTION	3
PRINT PARAMETERS	4

Enter Password, press **ENTER <↵>** to continue.

Password Screen

Enter Password:
Press ↵ to continue.

Press **3**, **Emergency Program Modify**.

Modify Screen

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Press ENTER to continue	

Choose tests from the keypad for this group. When complete, press **ENTER <↵>**.

Emergency Screen

Tests
In Emergency program
INR APTT FIB
New entry press ENTER to store
Press ESC to cancel

Tests will cycle allowing for Parameter changes to be made.

See **Section 5** for programming changes.

Programming Screen

S I G M A - A M E L U N G	
KC 4 DELTA	
DATE: 12 Sep 2001	1
TIME: 10 : 08	2
PROGRAM SELECTION	3
PRINT PARAMETERS	4

4.3 Individual Programming

From the **MAIN MENU**:
Press **3**, **Program Selection**.

Main Menu

S I G M A - A M E L U N G	
KC 4 DELTA	
DATE: 12 Sep 2001	1
TIME: 10 : 08	2
PROGRAM SELECTION	3
PRINT PARAMETERS	4

Enter Password, press **ENTER<↵>**.

Password Screen

Enter Password:
Press ↵ to continue.

Press **4**, **Individual Program Modify**

Modify Screen

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Press ENTER to continue	

Choose tests from the keypad for this group. When complete, press **ENTER <↵>**.

Individual Screen

Tests
In Individual Programming
INR APTT FIB
New entry press ENTER to store
Press ESC to cancel

When complete, press **ENTER <↵>**. Tests will cycle for parameter changes.

See **Section 5** for programming information.

Programming Screen

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Press ENTER to continue	

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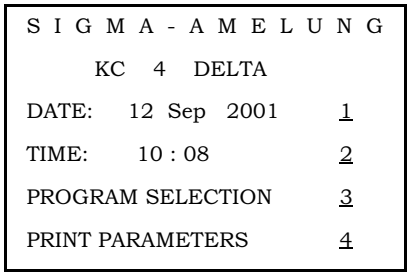
5.1 INR

The INR (International Normalized Ratio) is the preferred method for reporting Prothrombin Times in the United States. The PT percent activity curve and ratio are preferred in Europe.

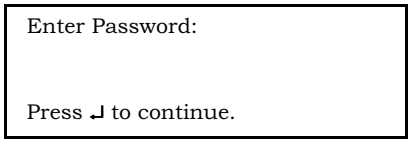
Allow the instrument to reach 37°C prior to testing.



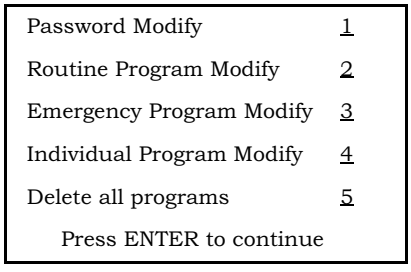
Prior to testing, a Test Program or profile needs to be established. The three programs available are discussed in **Section 4**. The Individual Program will be used here.

At the **MAIN MENU**,

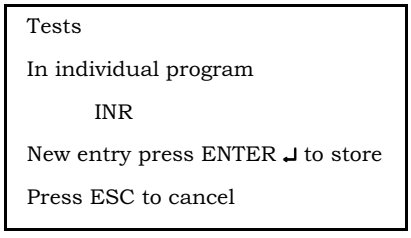
1. Press **3**, **Program Selection**.
2. Enter **4** digit Password:_ _ _ _;
3. Press <↵>.



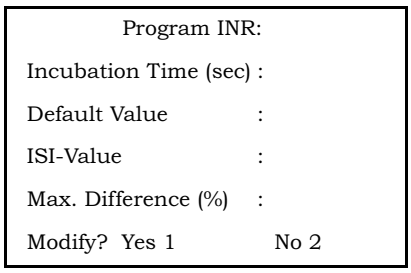
4. Select **Individual Program Modify 4**:



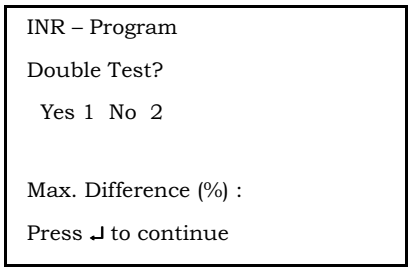
5. Choose tests from the keypad.
6. Press <↵> to store.



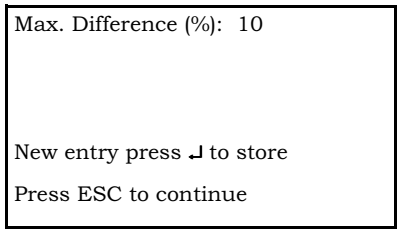
7. Select **Yes (1)** to change settings or select **No (2)** to leave as programmed.



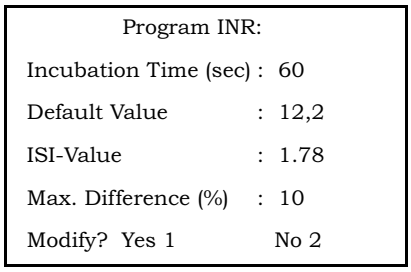
8. To test in duplicate, select **Yes (1)** or **No (2)** for single testing, press **<↵>**.



9. Enter the maximum difference allowed, where:
   Max difference (%) = (difference between values divided by the mean value) x 100



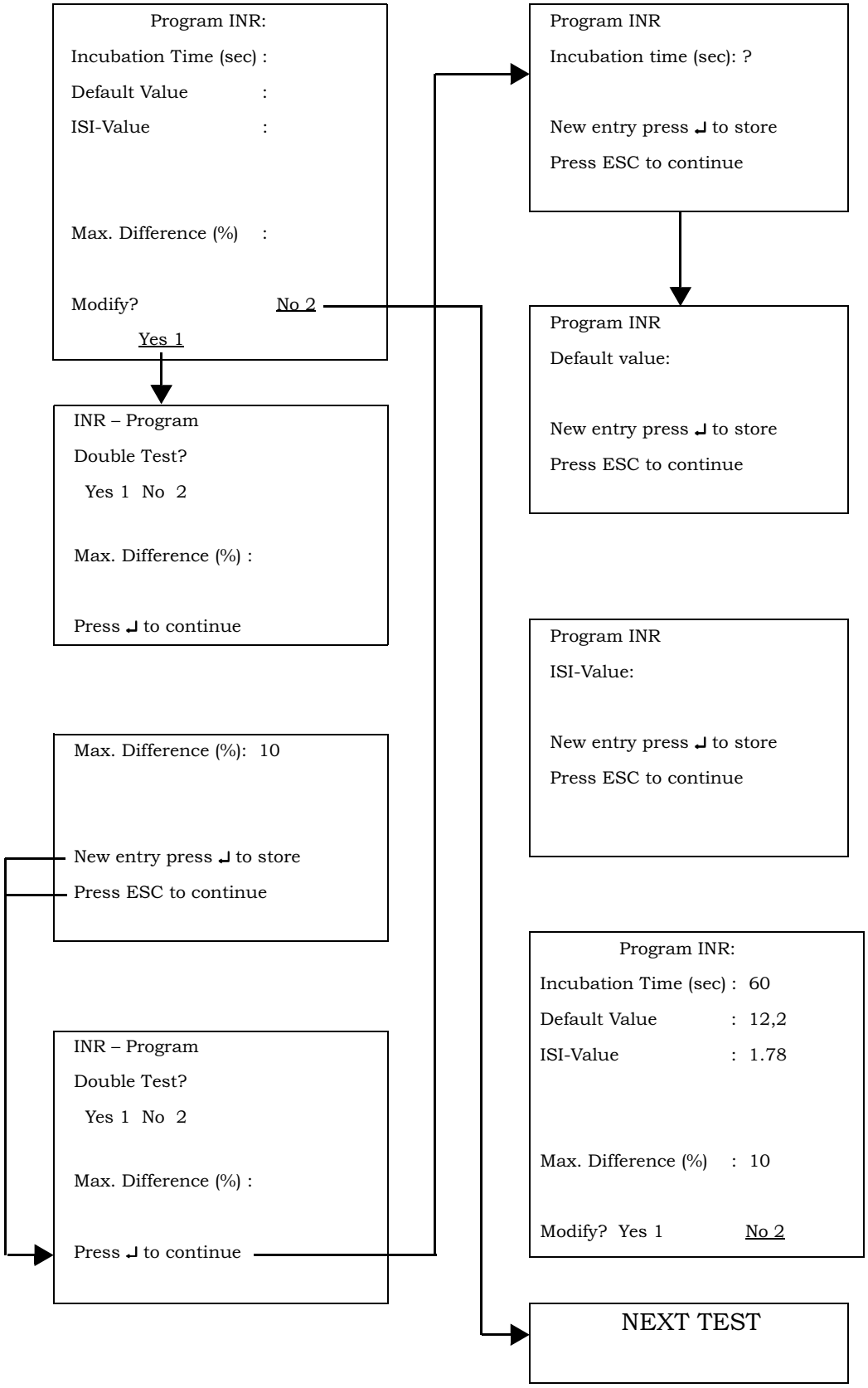
10. Program the incubation time referenced in the test application.
11. Default Value is the PT Mean Normal*
12. ISI Value for the thromboplastin in use.
13. Max. Difference as determined above.
14. Select **No (2)** when modification is complete. The cycle will return to the **MAIN MENU** when all modifications are complete.



The KC4 Δ™ will automatically calculate the INR using the programmed values entered for the mean of the Reference Range and the ISI value for the thromboplastin in use.

* Prior to entering values, a reference range should be determined for the lot number of thromboplastin that will be used. Because PT reference ranges can vary between laboratories and between different reagent formulations, determination of the reference range on the population being served is recommended. For accurate INR reporting, the determination of the geometric mean is recommended. When the geometric mean has been determined, values may be programmed into the KC4 Δ™.

5.2 INR Flow Chart



5.3 Prothrombin Time (Percent Activity Curve)

Allow the instrument to reach 37°C prior to testing.

S I G M A - A M E L U N G
KC 4 DELTA
DATE: 12 Sep 2001 1
TIME: 10 : 08 2
PROGRAM SELECTION 3
PRINT PARAMETERS 4

Prior to testing, a Test Program or profile needs to be established. The three programs available are discussed in **Section 4**. The **Individual Program** will be used here.

At the **MAIN MENU**:

1. Press **3**, **Program Selection**.
2. Enter **4** digit Password:_ _ _ _;
3. Press **<↵>**.

Enter Password:
Press ↵ to continue.

4. Select **Individual Program Modify 4**.

Password Modify	1
Routine Program Modify	2
Emergency Program Modify	3
Individual Program Modify	4
Delete all programs	5
Press ENTER to continue	

5. Choose tests from the keypad.
6. Press **<↵>** to store.

Tests
In individual program
PT
New entry press ENTER ↵ to store
Press ESC to cancel

7. Select **Yes (1)** to change settings or select **No (2)** to leave as programmed.

```
Program PT:
Incubation Time (sec) : ?
Calibration Curve PT  : ?
   Point 1  (%)       : ?
            (sec)     : ?
   Point 2  (%)       : ?
            (sec)     : ?
   Point 3  (%)       : ?
            (sec)     : ?
   PT MAX %           : ?
Difference Check PT   : No
      Modify?  Yes 1   No 2
```

8. Press **Yes (1)** if testing is to be done in duplicate, or **No (2)** if in single.

```
PT – Program
Double Test?
 Yes 1  No  2
 Max. Difference (%) :
Press ↵ to continue (more
parameters)
```

9. Enter the maximum difference allowed, where: Max difference (%) = (difference between values divided by the mean value) x 100

```
Max. Difference (%):  ?



New entry press ↵ to store
Press ESC to continue
```

10. Program the incubation time referenced in the test application.
11. Enter the reference value of the PT calibration curve. This is the first point, the highest point of the curve. This does not have to be 100%, it may vary depending on the source of the reference plasma.
12. Enter the clotting time in seconds of the first point of the PT calibration curve.

```
Program PT:
Incubation Time (sec) : 60
Calibration Curve PT  : ?
   Point 1  (%)       : 100
            (sec)     : 12
   Point 2  (%)       :
            (sec)     :
   Point 3  (%)       :
            (sec)     :
   PT MAX %           :
Max. Difference (%)   : 10
      Modify?  Yes 1   No 2
```

13. Enter the second value of the PT calibration curve. The second point, the middle point of the curve is a dilution of the reference plasma. **Example:** 30% is used.
14. Enter the clotting time in seconds for the middle point of the PT calibration curve.

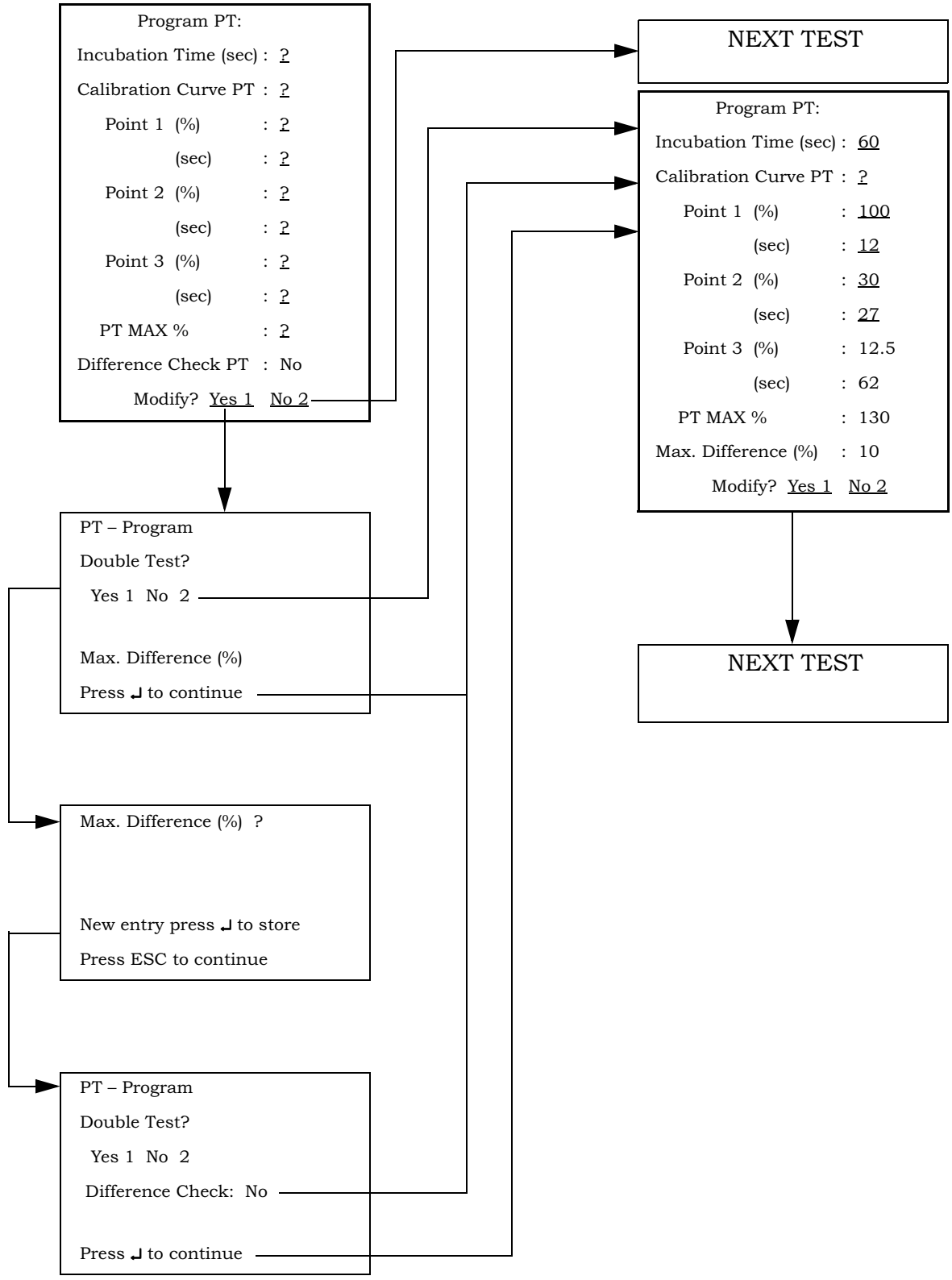
```
Program PT:
Incubation Time (sec) :  60
Calibration Curve PT  :  2
   Point 1  (%)       :  100
            (sec)     :  12
   Point 2  (%)       :  30
            (sec)     :  27
   Point 3  (%)       :
            (sec)     :
   PT MAX %           :
Max. Difference (%)   :  10
      Modify?  Yes 1   No 2
```

15. Enter the third value of the PT calibration curve. The third point, the lowest point of the curve is a dilution of the reference plasma. Example: 12.5% is used:
16. Enter the clotting time in seconds of the third point of the PT calibration curve.
17. Enter the Maximum % PT value. Values exceeding maximum value entered are not printed out. Example: 130% is used.

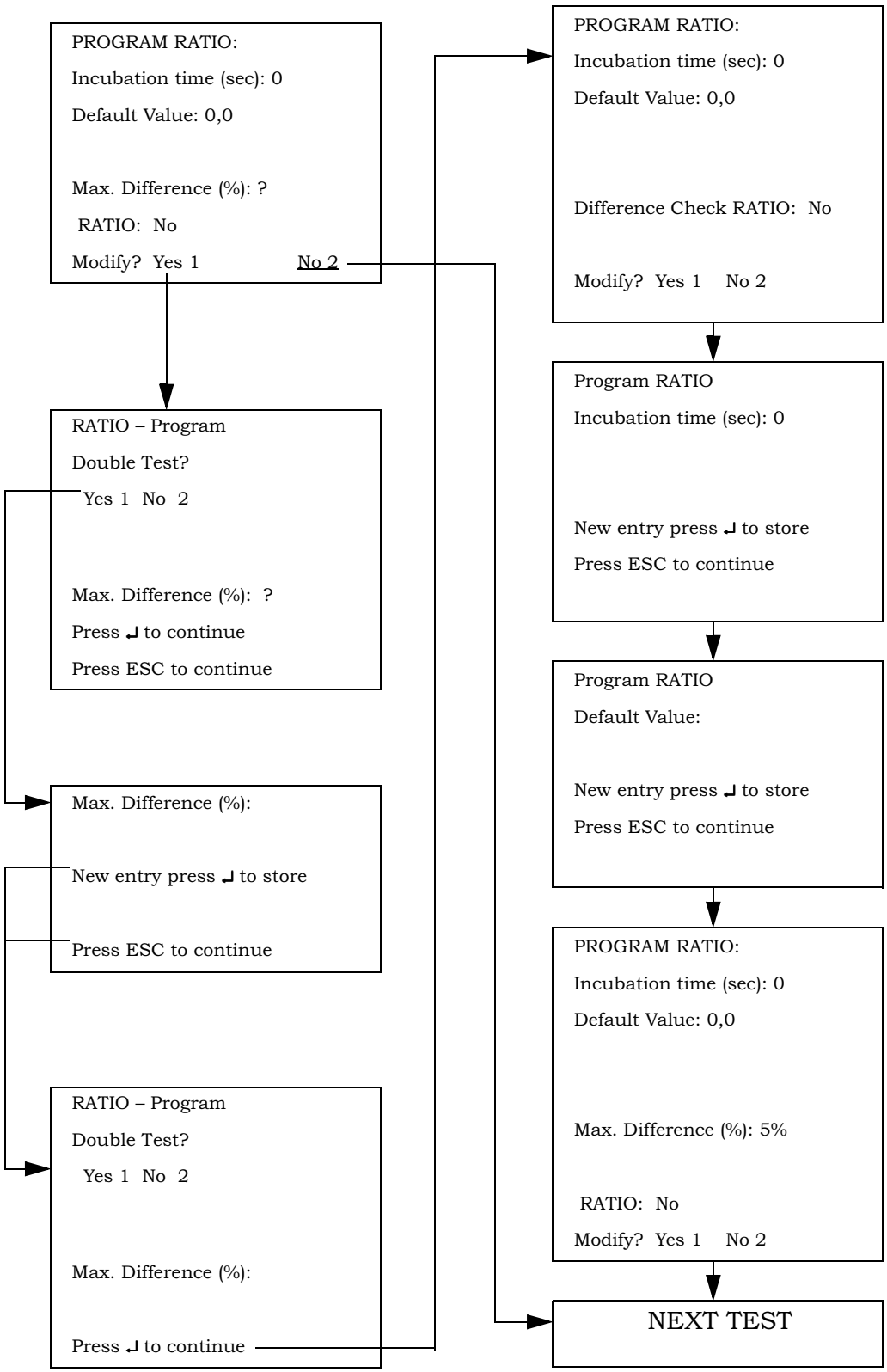
```
Program PT:
Incubation Time (sec) :  60
Calibration Curve PT  :  2
   Point 1  (%)       :  100
            (sec)     :  12
   Point 2  (%)       :  30
            (sec)     :  27
   Point 3  (%)       :  12.5
            (sec)     :  62
   PT MAX %           :  130
Max. Difference (%)   :  10
      Modify?  Yes 1   No 2
```

18. Select **No (2)**, when modification is complete. The cycle will return to the **MAIN MENU** when all modifications are complete.

5.4 PT (Percent Activity) Flow Chart



5.5 Ratio Flow Chart



5.6 Activated Partial Thromboplastin Time

Allow the instrument to reach 37°C prior to testing.

```
S I G M A - A M E L U N G
      KC  4  DELTA
DATE:   12 Sep 2001      1
TIME:     10 : 08        2
PROGRAM SELECTION        3
PRINT PARAMETERS         4
```

Prior to testing, a Test Program or profile needs to be established. The three programs available are discussed in **Section 4**. The **Individual Program** will be used here.

At the **MAIN MENU**:

1. Press **3**, **Program Selection**.
2. Enter 4 digit Password:_ _ _ _;
3. Press <↵>.

```
Enter Password:

Press ↵ to continue.
```

4. Press **Individual Program Modify 4**.

```
Password Modify              1
Routine Program Modify       2
Emergency Program Modify     3
Individual Program Modify    4
Delete all programs          5
    Press ENTER to continue
```

5. Choose tests from the keypad.
6. Press <↵>.

```
Tests
In individual program
        APTT
New entry press ENTER ↵ to store
Press ESC to cancel
```

7. Select **Yes (1)** to change settings or select **No (2)** to leave as programmed.

```
Program – PTT
Incubation time (sec): 240

Max. Difference (%): ?
Modify?  Yes  1   No  2
```

8. To test in duplicate, select **Yes (1)** or Select **No (2)** to test in single.

```
PTT – program
Double Test?
 Yes 1 No 2
Max. Difference (%): ?
Press ↵ to continue
```

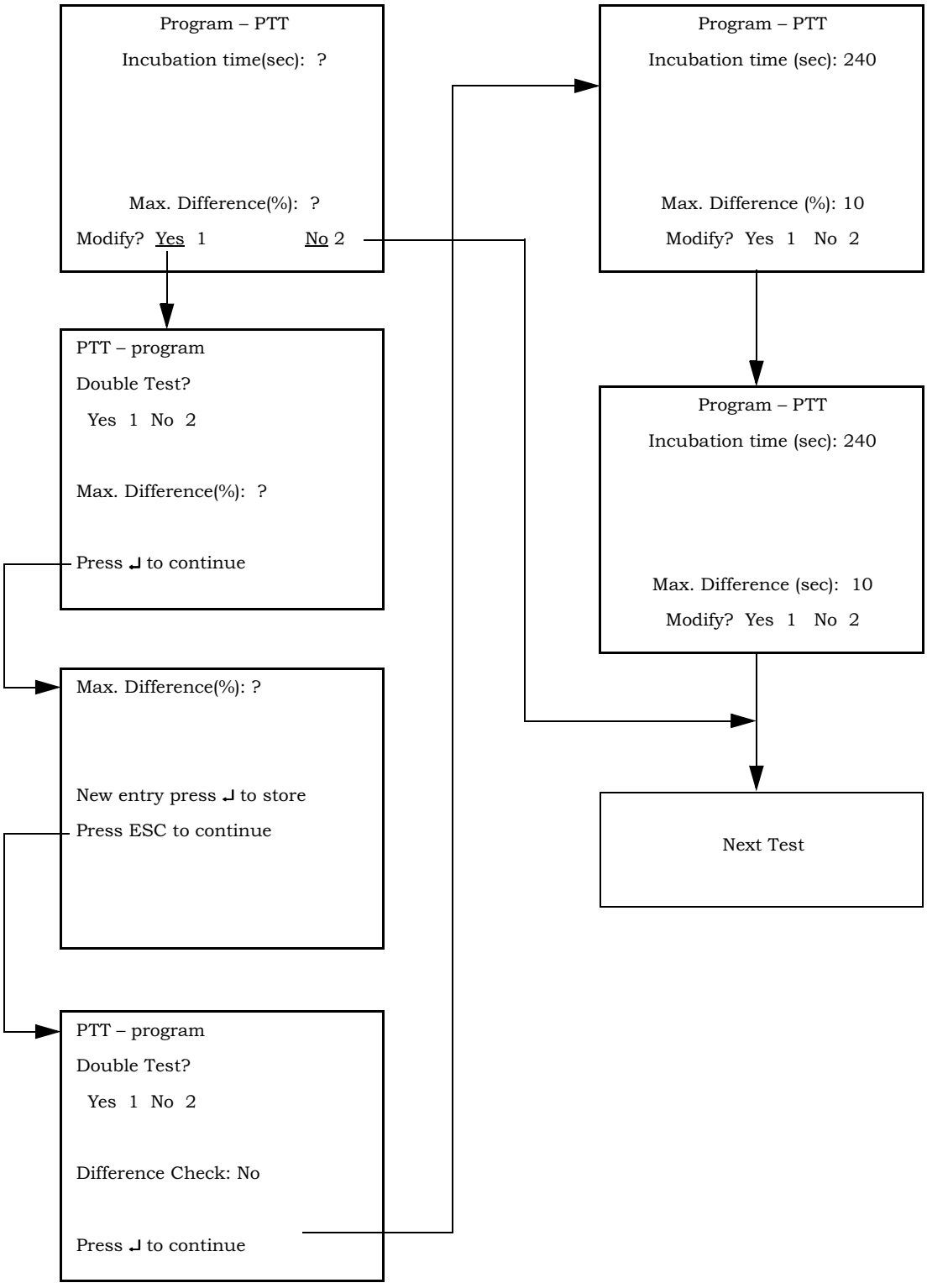
9. Enter the maximum value allowed, where:
   Max difference (%) = (difference between values divided by the Mean value) x 100.

```
Max. Difference (%):  10

New entry press ↵ to store
Press ESC to continue
```

10. Program the incubation time referenced in the test application.
11. Select **No (2)** when modifications are complete. The cycle will return to the **Main Menu** when all modifications are complete.

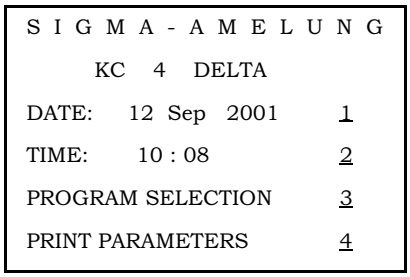
5.7 APTT Flow Chart



5.8 FIBRINOGEN

Prior to programming Fibrinogen, the concentration of the calibration curve points and the clotting time for each point must be known.

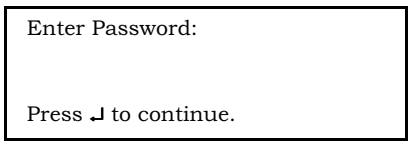
Allow the instrument to reach 37°C prior to testing.



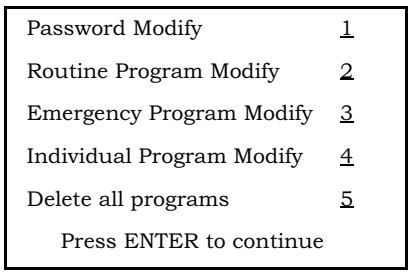
Prior to testing, the test must be programmed into a testing format as discussed in **Section 4**. The **Individual Program** will be used here.

At the **MAIN MENU**:

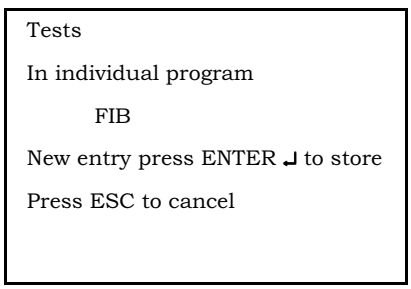
1. Press **3**, for **Program Selection**.
2. Enter 4 digit Password:_ _ _ _.
3. Press <↵>.



4. Press Individual Program Modify **4**.



5. Choose tests from the keypad.
6. Press <↵>.



7. To modify, select **Yes (1)** to change or, **No (2)** to test in leave as programmed.

```
Program – FIB:
Incubation Time (sec) :
Calibration Curve FIB:
   Point 1  (G/L)     :
            (sec)     :
   Point 2  (G/L)     :
            (sec)     :
   Point 3  (G/L)     :
            (sec)     :
Dilution              1:5
Max. Difference (%)   :
Modify?  Yes 1   No 2
```

8. Press **Yes (1)** if testing is to be done in duplicate or Press **No (2)** if in single.

```
FIB – Program
Double Test?
  Yes 1  No  2

 Max. Difference (%) :
Press ↵ to continue
```

9. Enter the maximum difference allowed, where: Max difference (%) = (difference between values divided by the Mean value) x 100

```
Max. Difference (%):


New entry press ↵ to store
Press ESC to continue
```

10. Press <↵> to continue changing parameters.

```
FIB – Program
Double Test?
  Yes 1  No  2

 Max. Difference (%) :
Press ↵ to continue
```

11. Choose the incubation time referenced in the test application.
12. Enter the reference value of the Fibrinogen reference plasma in G/L for the first point of the curve. This can vary depending on the source of the reference plasma. When using the KC4 Δ™ Coagulation Analyzer, care should be taken that the clot time of the highest point is more than the minimum read time (4.5 sec.) of the instrument. This can be achieved by utilizing the optimal dilutions for the reference plasma referred to in the reagent applications.

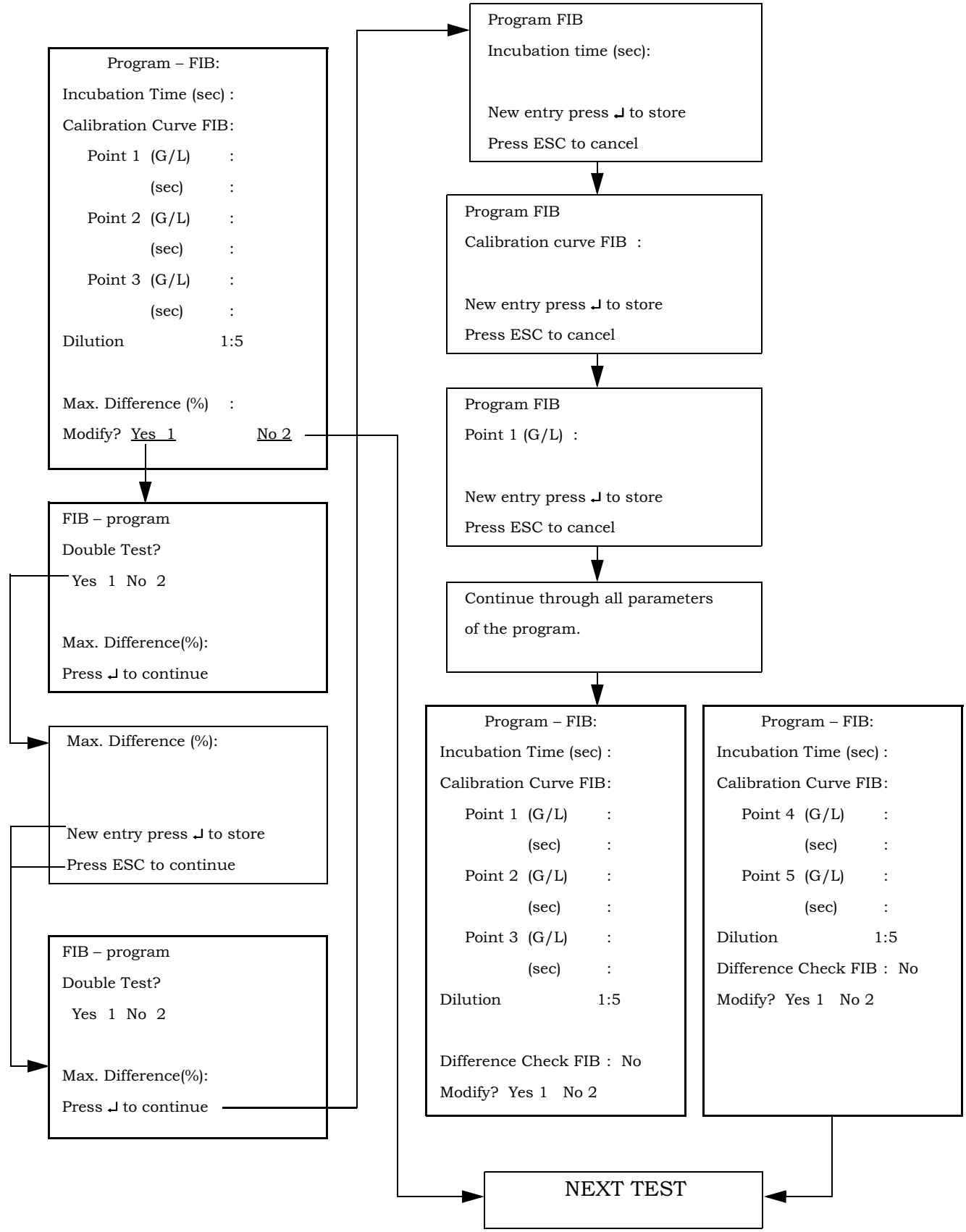
Program – FIB:	
Incubation Time (sec) :	
Calibration Curve FIB:	
Point 1 (G/L)	:
(sec)	:
Point 2 (G/L)	:
(sec)	:
Point 3 (G/L)	:
(sec)	:
Dilution	1:5
Max. Difference (%)	:
Modify? Yes 1 No 2	

Program – FIB:	
Incubation Time (sec) :	
Calibration Curve FIB:	
Point 4 (G/L)	:
(sec)	:
Point 5 (G/L)	:
(sec)	:
Dilution	1:5
Difference Check FIB : No	
Modify? Yes 1 No 2	

13. Enter the clotting time in seconds for the first point of the Fibrinogen reference curve.
14. Enter the second value for the Fibrinogen reference curve. The second point is a dilution of the reference plasma.
15. Enter the clotting time in seconds for the second point of the Fibrinogen reference curve.
16. Enter the third value for the Fibrinogen reference curve. The third point is a dilution of the reference plasma.
17. Enter the clotting time in seconds for the third point of the Fibrinogen reference curve.
18. Enter the fourth value of the Fibrinogen reference curve. The fourth point is a dilution of the reference plasma.
19. Enter the clotting time in seconds for the fourth point of the Fibrinogen reference curve.
20. Enter the fifth value of the Fibrinogen reference curve. The fifth point is a dilution of the reference plasma.
21. Enter the clotting time in seconds for the fifth point of the Fibrinogen reference curve.
22. Enter the dilution for the patient or control samples to be analyzed. Fibrinogens use a 5 point reference curve.
23. Select **No (2)** when modifications are complete. The cycle will return to the **Main Menu** when all modifications are complete.

Note: Suggested dilutions and volumes used are found in the KC4 Δ™ Fibrinogen Application.

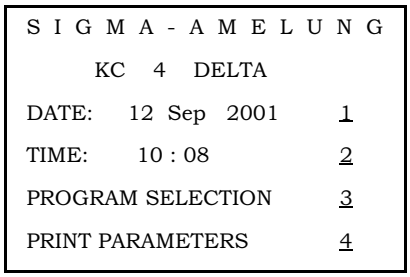
5.9 Fibrinogen Flow Chart



5.10 FACTORS

Prior to programming Factors, the concentration of the calibration curve points must be known and the clotting time for each point must be established.

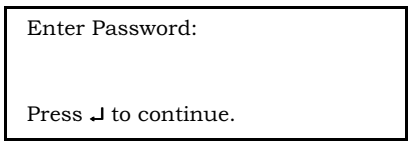
Allow the instrument to reach 37°C prior to testing.



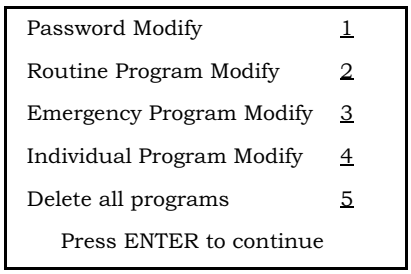
Prior to testing, a Test Program or profile needs to be established. The three programs available are discussed in **Section 4**. The **Individual Program** will be used here.

At the **MAIN MENU**:

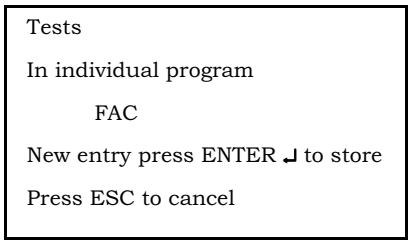
1. Press **3**, **Program Selection**.
2. Enter 4 digit Password:_ _ _ _.
3. Press **<↵>**.



4. Select **Individual Program Modify 4**.



5. Choose desired tests from the kepad.
6. Press **<↵>**.



7. Select **Yes (1)** to change settings or select **No (2)** to go to the next test.

```
PROGRAM   FAC  Factor = 0
Incubation Time (sec) :
Count Points          :
Calibration Curve   F ?
   Point 1  (%)       : 0,0
            (sec)     : 0,0
   Point 2  (%)       : 0,0
            (sec)     : 0,0
   Point 3  (%)       : 0,0
            (sec)     : 0,0
Modify?  Yes  1  No  2 (next test)
```

8. To test in duplicate, select **Yes (1)** or **No (2)** for single.

```
FAC – Program
Double Test?
  Yes  1  No  2

Max. Difference:  ?
Press ↵ to continue
```

9. Enter the maximum difference allowed, where: Maximum value (%) = (difference between values)/mean value x 100.

```
Max. Difference(%): 0
New entry press ↵ to store
Press ESC to continue
```

10. Press <↵> to continue.

```
FAC - Program
Double Test?
  Yes  1  No  2

Max. Difference:  ?
Press ↵ to continue
```

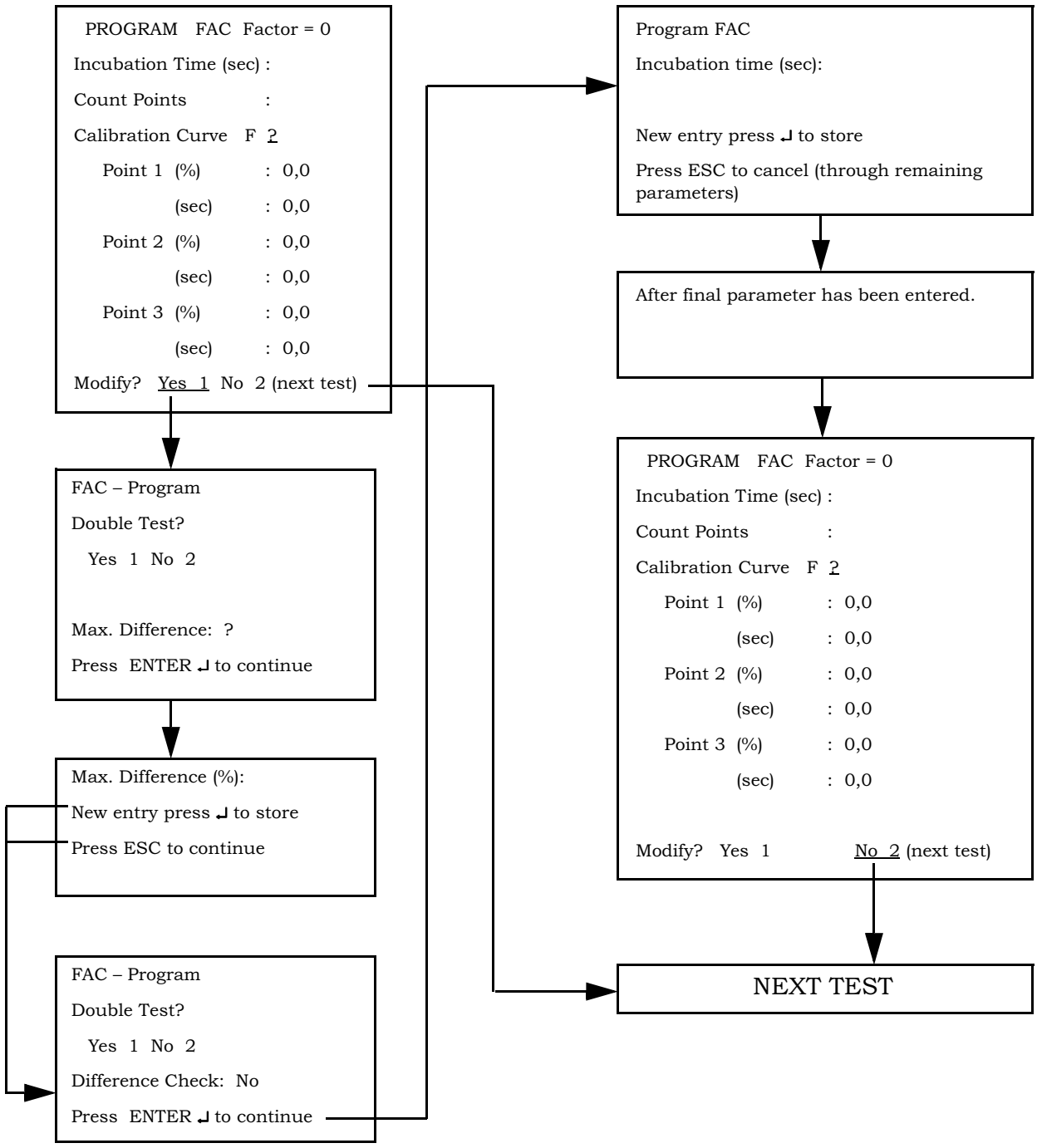
Note: Only one factor curve may be entered at a time.

11. Enter the Factor that will be used.
12. Enter an incubation time referenced in the test application.
13. Choose as many Curve Points (Count Points) as appropriate for testing requirements (9 points max).
14. Enter the reference value of the Factor calibration curve. This is the first point, the highest point of the curve. This does not always have to be 100%, it can vary depending on the source of the reference plasma.
15. Enter the clotting time in seconds of the first point of the Factor calibration curve.
16. Continue entering percentage points and seconds to complete the calibration curve.

PROGRAM FAC Factor = 0
Incubation Time (sec) :
Count Points :
Calibration Curve F ?
Point 1 (%) : 0,0
(sec) : 0,0
Point 2 (%) : 0,0
(sec) : 0,0
Point 3 (%) : 0,0
(sec) : 0,0
Modify? Yes 1 No 2 (next test)

17. Select **No (2)** when modifications are complete. The cycle will return to the **Main Menu** when all modifications are complete.

5.11 Factors Flow Chart


PROGRAM FAC Factor = 0
Incubation Time (sec) :
Count Points :
Calibration Curve F 2
Point 1 (%) : 0,0
(sec) : 0,0
Point 2 (%) : 0,0
(sec) : 0,0
Point 3 (%) : 0,0
(sec) : 0,0
Modify? Yes 1 No 2 (next test)
FAC – Program
Double Test?
Yes 1 No 2
Max. Difference: ?
Press ENTER ↵ to continue
Max. Difference (%):
New entry press ↵ to store
Press ESC to continue
FAC – Program
Double Test?
Yes 1 No 2
Difference Check: No
Press ENTER ↵ to continue
Program FAC
Incubation time (sec):
New entry press ↵ to store
Press ESC to cancel (through remaining parameters)
After final parameter has been entered.
PROGRAM FAC Factor = 0
Incubation Time (sec) :
Count Points :
Calibration Curve F 2
Point 1 (%) : 0,0
(sec) : 0,0
Point 2 (%) : 0,0
(sec) : 0,0
Point 3 (%) : 0,0
(sec) : 0,0
Modify? Yes 1 No 2 (next test)
NEXT TEST

5.12 STATS

STATS are programmed in **Section 4.2 Emergency Program**.

1. From the **MAIN MENU** or if **ROUTINE** testing is in progress, press **<↵>** to bring up the Operating screen:
2. From the keypad press **<RUN>**.
3. At the **Test Selection Screen**, choose **Emergency Program (2)** to bring up the **Operating Screen**. If 2 beeps sound, nothing has been programmed; see **Section 4**.
4. If multiple tests are grouped in the STAT Mode; press **<↵>** to change to other tests in the group.

Regularly performed quality control is the best monitor of test performance. To assure that control and unknown sample results are evaluated under the same test conditions, control material should be included with each run.

The reagent manufacturer's QC recommendations should be used as a guide for establishing a QC protocol. Control results deviating from established ranges are indicative of a system failure and should be investigated immediately. The more common sources of error and the corrective action to take are presented in the Troubleshooting section, **Section 8**.

Introduction

There is no routine mechanical maintenance associated with the KC4 Δ™. The KC4 Δ™ was factory calibrated for rotational speed, magnetic sensor strength and temperature.

General housekeeping is the only maintenance that need be performed with any regularity. Occasional cleaning with a damp paper towel is recommended to remove accumulated dust or other material. Spills should be cleaned up as they occur.

Reagents can be corrosive and any spillage into the reagent incubation well should be cleaned up immediately. All sample spills should be considered to have created a potentially biohazardous environment and should be cleaned up immediately using appropriate safeguards to avoid personal contamination. If decontamination is required, wipe area with a paper towel moistened with a mild disinfectant.

Any balls that inadvertently find their way into the bottom of any of the wells can be removed using a magnet.

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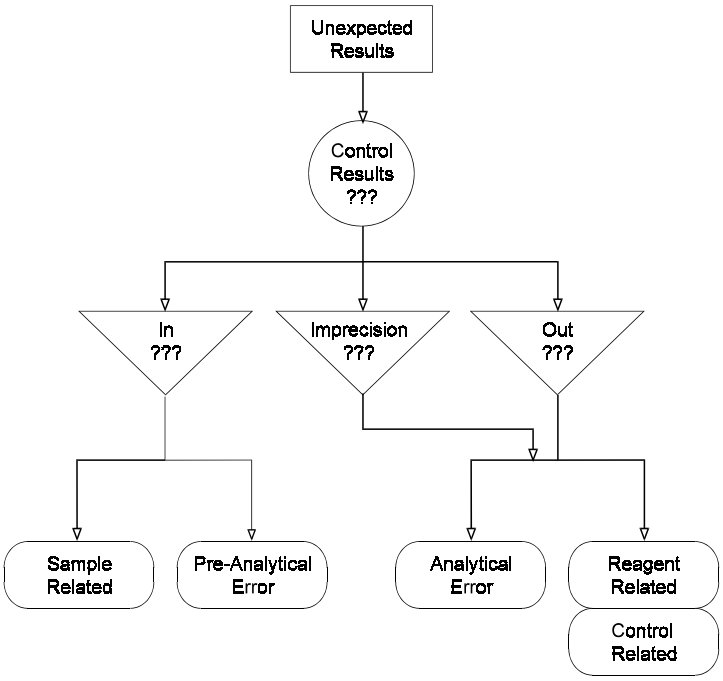
8.1 Troubleshooting Flow Diagram



8.2 Troubleshooting Procedures Table

Symptoms	Possible Causes	Corrective Action
After **pressing On/ Off**, the display is empty; measurement well is not rotating.	**Instrument Error** KC4 Δ™ not connected to power supply or power supply not connected to outlet.	Assure that the connecting cable is seated firmly in the power supply socket. Assure that the power supply cord is connected to the appropriate outlet.
After **pressing On/ Off**, temperature fails to stabilize at 37°C; measurement well is rotating.	**Instrument Error** Nonfunctional sensor or thermostat overheating.	Place approximately 3 ml water into a 15 mm test tube. Insert a thermometer. Observe temperature after a 10–15 minute equilibration period.
		Call Sigma Diagnostics Technical Service 1-800-325-0250.
Controls are in range Unexpected results on patient samples.	**Pre-Analytical error** Overfill or underfill of collection tube.	Full draw in commercial vacuum tubes assures correct blood/anticoagulant ratio.
	Pre-Analytical error Incorrect volume, type (e.g., EDTA, heparin), concentration or lack of anticoagulant.	Use anticoagulant as recommended by the reagent manufacturer.

Symptoms	Possible Causes	Corrective Action
(continued) **Controls are in range** Unexpected results on patient samples.	**Pre-Analytical error** Ratio of anticoagulant to blood is inappropriate.	Failure to correct citrate volume for patients with high (>55%) or low (<21%) hematocrit.
	Pre-Analytical error Clotted specimen.	Testing should never be performed on specimens containing micro or macro clots.
	Pre-Analytical error Inadequate or too vigorous mixing of specimen.	Invert gently to mix well without mechanical trauma.
	Pre-Analytical error Contamination with heparin.	Do not draw blood from a heparin lock or any other heparinized line.
	Pre-Analytical error Delay in transporting or processing or the use of nonstandardized procedures for transporting, processing, storing or testing the specimen.	Follow manufacturer's instructions.
		Centrifuge immediately. Centrifuge at correct rcf for correct time interval.
		Store no longer than 4 hours at room temperature or refrigerated temperatures.
	Pre-Analytical error Contact with glass.	Transfer plasma to plastic storage tube using plastic transfer pipettes.
	Sample related Loss of Factors V and VIII	Avoid heating at 37 °C for longer than 5 minutes.
Controls out of range Unexpected results.	**Sample related** Incorrect volume being used.	Follow manufacturer's instructions.
	Reagent related Contaminated reagents.	Reconstitute new reagent or open new bottle.
	Reagent related Wrong reagent being used.	Follow manufacturer's instructions.
	Reagent related Incorrect volume of reagent being used.	Follow manufacturer's instructions.
	Reagent related Reconstitution with incorrect diluent volume.	Follow manufacturer's instructions.
	Reagent related Reconstitution with other than the recommended diluent.	Follow manufacturer's instructions.

Symptoms	Possible Causes	Corrective Action
(continued) **Controls out of range** Unexpected results.	**Reagent related** New lot number of reagent with different reactivity.	Lot-to-lot variation in reagent reactivity is not unusual. Re-verify reference range. Prepare new reference curves as appropriate.
	Reagent related Reagent defective due to mishandling in shipping or storage.	First use of reagent in this shipment? Temperature of storage area appropriate?
	Reagent related Reagent deteriorated.	Do not use reagent beyond stated reconstituted stability time or beyond expiration date of un-reconstituted reagent.
	Reagent related Reagent deteriorated because of extended warming in reagent well.	Do not store reagent on instrument. At the completion of testing, remove reagent from instrument, cap and store according to the manufacturer's instructions.
	Reagent related Reagent contaminated.	Avoid contact of pipette tips with previously pipetted sample or reagent.
	Control related Deteriorated or contaminated control material.	Prepare fresh controls
		Incorrectly reconstituted control material(s). Reconstitute according to the manufacturer's instructions. Use only fresh deionized water for reconstitution.
	Analytical error Incorrect incubation time.	Follow manufacturer's instructions.
	Analytical error Incorrect reagent temperature.	15 mm tube must be used. No more than 3.5 ml of reagent should be placed in tube. Allow 15–20 minutes for reagent to come to temperature in the reagent well. Some reagents, (Thrombin Reagent for Fibrinogen) should not be warmed but must be equilibrated to room temperature prior to use. Follow reagent manufacturer's instructions.
	Analytical error Incorrect testing sequence.	Follow manufacturer's instructions.

Symptoms	Possible Causes	Corrective Action
Erratic within-run test results. **Controls may be in or out of range.**	**Analytical error** Imprecision in manual pipetting of sample and reagent.	Perform pipette maintenance. A pipette Operating Manual is included in the box of the automatic pipette provided with the KC4 Δ™.
		Practice pipetting technique. Refer to the pipetting section for guidance.
		Incorrect dispense position. Consistent location for reagent dispense is important. Refer to Pipetting section for guidance.
		Failure to mix particulate reagent prior to use. Cover top of tube with cap or Parafilm™ and invert gently to mix.
		Failure to mix sample and first reagent. After dispense of sample and reagent, pick the cuvette up and swirl gently 5–6 times to evenly disperse the mixture in the bottom of the cuvette.
	Reagent related Inconsistent or inaccurate reconstitution of reagent or control material.	
	Reagent related Reagent deterioration caused by extended heating in reagent well.	Reconstitute new reagent and/ or control material.
	Reagent related Reagent concentration caused by evaporation.	Remove reagent from instrument at the completion of testing.

Symptoms	Possible Causes	Corrective Action
(continued) Erratic within-run test results. **Controls may be in or out of range.**	**Sample related** Improper specimen collection and handling.	Reagents should be capped when not in use.
		Check sample integrity looking for microclots, hemolysis or other irregularities.
		Assure that ratio of anticoagulant to sample is correct (full draw).
		Draw a new specimen. If erratic results are again obtained, inquire about the clinical condition of the patient. Results on patients in DIC are characteristically erratic.
	Sample related No sample added.	Observe recommended sample storage conditions.
No clot formation	**Sample related** Low fibrinogen.	Assure that sample has been added.
	Reagent related No or incorrect reagent added.	A deficiency of Fibrinogen will greatly prolong the results of many coagulation tests.
	Analytical error No ball in cuvette.	Assure that correct reagents are being used.
Clot formed but not detected. Timer does not stop.	**Analytical error** Cuvette not seated in well.	Assure that the ball has not fallen out of the cuvette prior to insertion into measuring well.
	Sample related. Clot formed before 4.6 seconds.	Ball is positioned above the sensor. Assure that there are no balls or other obstructing material in the bottom of the well.
		If performing Fibrinogen, use next higher dilution.
		To stop the timer, insert a new cuvette with ball in the measuring well. After 10 seconds, lift the cuvette out of the well.

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A.1 INR Fast Track

Note: Use the Multipette. See Section 5 for information regarding programming.

1. From the **MAIN MENU**, press **<↵>**.
2. Press **<RUN>**.
3. Select **Individual Program**.
4. Select **<INR>** and press **<↵>**.
5. Enter Samples per rack (the test must be set up for **duplicates**; so the count would be 1 for a single sample or 2 for 2 samples).
   Press **<↵>**.
6. Enter Patient ID and press **<↵>**.
7. Warm **thromboplastin** reagent to **37°C**.
8. Place cuvettes into rack positioned in the unheated preparation area.
9. Dispense one ball into each cuvette if not using a Tetravette.
10. Pipette 50 µl sample into bottom of each cuvette.
11. Close flap, remove rack from preparation area and swirl gently 4–5 times.
12. Transfer rack into heated incubation area or into the rotating test positions.
13. Incubate for a minimum of **60 seconds** up to a maximum of **180 seconds**.
14. Press to prepare **Incubation Timers** and then press for each channel with sample in cuvette.
15. Upon completion of incubation Timing for the last well being tested, place rack into rotating test positions, and open Flap.
16. Press **<START>** and begin dispensing PT reagent into wells. With the Multipette, the timer starts automatically as reagent is dispensed.
17. Record results.

A.2 APTT Fast Track

Note: Use the Multipette. See Section 5 for information regarding specific programming.

1. From the **MAIN MENU**, press **<↵>**.
2. Press **<RUN>**.
3. Select **Individual Program**.
4. Select **<APTT>** and press **<↵>**.
5. Enter Samples per rack (the test must be set up for **duplicates;** the count would be 1 for a single sample or 2 for 2 samples).
   Press **<↵>**.
6. Enter Patient ID and press **<↵>**.
7. Warm **APTT** reagent and **$CaCl_2$** to **37°C**.
8. Place cuvettes into rack positioned in the unheated preparation area.
9. Dispense one ball into each cuvette if not using a Tetravette.
10. Pipette 50 µl sample into bottom of each cuvette.
11. Close flap, remove rack from preparation area, and swirl 4–5 times.
12. Transfer rack to heated incubation area or into the rotating test positions.
13. Incubate for **240 seconds** (This is the **TOTAL** incubation time for the test; it is a combination of the sample warm-up plus the warming of the **APTT** reagent).
14. Press ⏲ to prepare **Incubation Timers** and then press ⏻ for each channel with sample in cuvette.
15. At **185 seconds** add **50 µl APTT** reagent.
16. Upon completion of incubation Timing for the last well being tested, place rack into rotating test positions, and open flap.
17. Press **<START>**. Begin dispensing **50 µl $CaCl_2$** into cuvettes. Using the Multipette, measurement timing begins automatically.
18. Record results.

A.3 FIBRINOGEN Fast Track

Note: Use the Multipette. See Section 5 for information regarding programming.

1. From the **MAIN MENU**, press **<↵>**.
2. Press **<RUN>**.
3. Select **Individual Program**.
4. Select **<FIB>** and press **<↵>**.
5. Enter Samples per rack (the test must be set up for **duplicates;** the count would be 1 for a single sample or 2 for 2 samples).
   Press **<↵>**.
6. Enter Patient ID and press **<↵>**.
7. Warm **Thrombin Reagent** to room temperature **(18–25°C)**.
8. Prepare 1:10 sample dilutions.
9. Dispense one ball into each cuvette if not using a Tetravette.
10. Pipette **100 µl diluted sample** (patient test plasmas are prepared by diluting plasma 1:10 with **Imidazole Buffer***) into bottom of each cuvette.
11. Close flap, remove rack from preparation area and swirl gently 4–5 times.
12. Transfer rack into heated incubation area or into the rotating test positions.
13. Incubate for a minimum of **60 seconds** and up to a mzximum of **180 seconds**.
14. Press ⏲ to prepare **Incubation Timers** and then press ⏻ for each channel with sample in cuvette.
15. Upon completion of incubation timing for the last well being tested place rack into rotating test positins, and open flap.
16. Press **<START>** and begin dispensing **100 µl Thrombin Reagent** into each well. Using the Multipette, the timer starts automatically as reagent is dispensed.
17. Record results.

*Refer to test application.

A.4 FIBRINOGEN CALIBRATION CURVE DILUTION

Fibrinogen Reference Buffer Dilution	(ml)	(ml)	Dilution Factor
1:7	0.1	0.6	1.43
1:8	0.1	0.7	1.25
1:10	0.1	0.9	1
1:20	0.1	1.9	0.5
1:25	0.1	2.4	0.4
1:30	0.1	2.9	0.33
1:40	0.1	3.9	0.25

A.5 EXTRINSIC FACTORS II, V, VII and X FAST TRACK

Note: Use the Multipette. See Section 5 for information regarding programming.

1. From the **MAIN MENU**, press **<↵>**.
2. Press **<RUN>**.
3. Select **Individual** Program.
4. Select **<FAC>** and press **<↵>**.
5. Enter Samples per rack (the test must be set up for **duplicates;** the count would be 1 for a single sample or 2 for 2 samples).
   Press **<↵>**.
6. Enter Patient ID and press **<↵>**.
7. Warm **thromboplastin** Reagent to **37°C**.
8. Prepare 1:10 sample dilutions.
9. Dispense one ball into each cuvette if not using a Tetravette.
10. Pipette **50 µl diluted sample** (patient test plasmas are prepared by diluting plasma 1:10 with **Imidazole Buffer**)* into bottom of each cuvette.
11. Add 50 **µl** appropriate **Factor Deficient Plasma**.
12. Close flap, remove rack from preparation area and swirl gently 4–5 times.
13. Transfer rack into heated incubation area or into the rotating test positions.
14. Incubate for a minimum of **60 seconds** and up to a maximum of **180 seconds**.
15. Press to prepare **Incubation Timers** and then press for each channel with sample in cuvette.
16. Upon completion of incubation timing for the last well being tested, place rack into rotating test positions, and open flap.
17. Press **<START>** and begin dispensing **100 µl thromboplastin** into each well. Using the Multipette, the timer starts automatically as reagent is dispensed.
18. Record results.

* Refer to test application.

A.6 EXTRINSIC FACTOR STANDARD CURVE DILUTIONS

Dilution	Reference Plasma (ml)	Buffer (ml)	Dilution Factor
1:10	0.2	1.8	1
1:20	1.0 of 1:10	1.0	0.5
1:40	1.0 of 1:20	1.0	0.25
1:80	1.0 of 1:40	1.0	0.125
1:160	1.0 of 1:80	1.0	0.0625

A.7 INTRINSIC FACTORS VIII, IX, XI and XII FAST TRACK

Note: Use the Multipette. See Section 5 for information regarding programming.

1. From the **MAIN MENU**, press <↵>.
2. Press **<RUN>**.
3. Select **Individual Program**.
4. Select **<FAC>** and press <↵>.
5. Enter Samples per rack (the test must be set up for **duplicates;** the count would be 1 for a single sample or 2 for 2 samples).
   Press <↵>.
6. Enter Patient ID and press <↵>.
7. Warm **APTT** reagent and **$CaCl_2$** to **37°C**.
8. Prepare 1:5 sample dilutions.
9. Place cuvettes into rack positioned in the unheated preparation area.
10. Dispense one ball into each cuvette if not using a Tetravette.
11. Pipette **50 µl diluted sample** (patient test plasmas are prepared by diluting plasma 1:10 with **Imidazole Buffer**)* into bottom of each cuvette.
12. Add **50 µl** appropriate **Factor Deficient Plasma**.
13. Close flap, remove rack from preparation area and swirl gently 4–5 times.
14. Transfer rack into heated incubation area or into the rotating test positions.
15. Incubate for **240 seconds** (This is the **TOTAL** incubation time for the test, a combination of the sample warm-up plus the warming of the APTT reagent).
16. Press to prepare **Incubation Timers** and then press for each channel with sample in cuvette.
17. At **185 seconds** add **50** µl **APTT** reagent.
18. Upon completion of incubation timing for the last well being tested, place rack into rotating test positions, and open flap.
19. Press **<START>**. Begin dispensing **50 µl $CaCl_2$** into cuvettes. Using the Multipette, measurement timing begins automatically.
20. Record results.

* Refer to test application

A.8 INTRINSIC FACTOR STANDARD CURVE DILUTIONS

Dilution	Reference Plasma (ml)	Buffer (ml)	Dilution Factor
1:5	0.4	1.6	1
1:10	1.0 of 1:5	1.0	0.5
1:20	1.0 of 1:10	1.0	0.25
1:40	1.0 of 1:20	1.0	0.125
1:80	1.0 of 1:40	1.0	0.0625